Detection and classification of gestational trophoblastic neoplasia

Yalck Eysbouts

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Chapter 1

Introduction and thesis outline

GESTATIONAL TROPHOBLASTIC DISEASE

Gestational trophoblastic disease (GTD) represents a heterogeneous group of pregnancy-related disorders, consisting of the premalignant hydatidiform mole and the malignant disorders invasive mole, choriocarcinoma, placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT) [1,2]. The term gestational trophoblastic neoplasia (GTN) has been applied collectively to the malignant counterparts [3]. More recently, two tumor-like trophoblastic lesions have been described: exaggerated placental site reaction and placental site nodule [4,5]. These lesions are generally incidental findings in uterine curettages, biopsies or occasionally in hysterectomy specimens [6,7]. Following these lesions, local recurrence or progression to gestational trophoblastic neoplasia is exceptional, therefore no specific treatment or follow-up is usually necessary [8].The relevance of the exaggerated placental site reaction and placental site nodule consists of possible misdiagnosis of PSTT and ETT. Placental site nodules have been described as the benign counterparts of ETT, whereas exaggerated placental site lesions show comparison with PSTT [4,9].

Suction curettage is the preferred method of evacuation in patients with suspected hydatidiform mole, irrespective of uterine size [10,11]. In developed countries, due to the introduction of routine ultrasonography in the early 1990s, patients with hydatidiform moles are typically diagnosed in the first trimester of pregnancy, when symptoms are usually absent [12]. Hydatidiform moles can be divided into complete and partial hydatidiform mole. Complete hydatidiform moles (CHM) are diploid and androgenetic, resulting from duplication of the haploid genome of a single sperm or dispermic fertilization of an ovum (Figure 1). In either case, maternal chromosomes are lost and nuclear DNA is entirely paternal [2,4]. Complete hydatidiform moles are characterized by uniform enlarged villi, diffuse hyperplasia and varying degrees of atypia in the absence of an ascertainable fetus [6,7]. In contrast, partial hydatidiform moles (PHM) usually have a triploid karyotype and arise from dispermic fertilization of a haploid ovum. Fetal parts may be present and villous changes are more patchy and scattered [4,13,14]. In some cases, distinction between PHM, CHM and non-molar hydropic abortions can be challenging [13,15,16]. The subdivision between complete and partial hydatidiform moles is of interest, since the malignant potential of both entities differs. Post-molar GTN occurs in approximately 15-20% of complete hydatidiform moles and 0.5-1% of partial hydatidiform moles and implies further treatment [1,10,17-19].

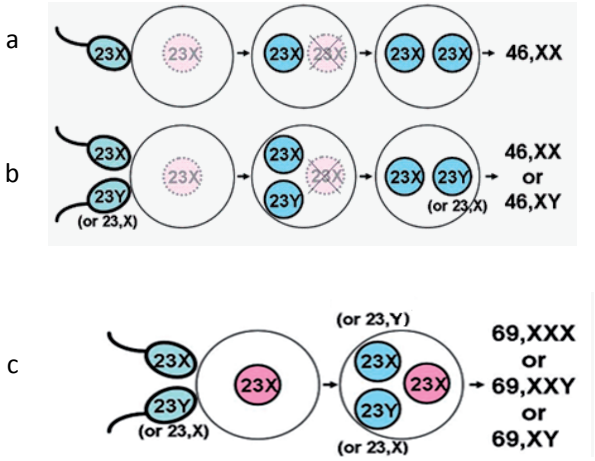

Figure 1. Cytogenetic origin of complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM). CHM develops after monospermic (a) or dispermic (b) fertilization of an ovum. Maternal chromosomes are lost before or just after conception, resulting in a diploid karyotype. PHM results from dispermic fertilization of a normal ovum (c). This generally results in a triploid karyotype[a].

[a] Adapted from Seckl MJ et al. Gestational trophoblastic disease: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up; *Annals of Oncology 24 (supplement 6):* Vi 39-vi 50, 2013;

In post-molar GTN, trophoblastic activity persists after evacuation of a hydatidiform mole, expressed by a plateau or rise in serum human chorionic gonadotropin (hCG) concentration [1,2]. The international Federation of Obstetrics and Gynecology (FIGO) defines post-molar GTN as: 1) a plateau in hCG concentration in 4 consecutive blood samples over a period of 3 weeks, 2) or a rise in hCG concentration for 3 consecutive samples over a period of 2 weeks [20-22]. An elevated but falling hCG 6 months after evacuation is no longer considered an absolute criterion as serum hCG normalization will generally occur in these patients [21,23].

INCIDENCE

Epidemiological studies have reported wide regional variations in the incidence of GTD [24]. Our group has previously reported a GTD incidence rate in the Netherlands of 1.56 per 1000 deliveries per year, with an incidence for hydatidiform moles of 1.34

per 1000 deliveries per year [25]. The incidence rates were derived from population-based data between 1995 and 2008. The incidence rate of HM was broadly comparable with recent findings of population-based studies in Europe and North-America [26-28]. Reported frequencies from Asian countries are highly heterogeneous with rates ranging from 1 to 3 per 1000 pregnancies in Japan, China and Korea to over 10 per 1000 pregnancies in Indonesia and India [26,27,29-32]. Lack of centralized databases, heterogeneity in case definition and inability to adequately describe the population at risk (e.g. the number of pregnancies, deliveries or life births in the population) have likely contributed to the wide variations in incidence rates worldwide [2,33-35].

In addition, although morphologically CHM and PHM have distinct histopathological features, the subjective nature of the morphological abnormalities give rise to inter-observer variation in diagnosis [36,37]. Especially in the present ultrasound era, when evacuation is usually performed at an early stage of pregnancy and classic morphological features will be less apparent [38,39]. The introduction of immune-staining with $p57^{kip2}$, an imprinted marker expressed only by the maternal genome and therefore absent in CHM but present in PHM and ploidy analysis such as flow cytometry can be helpful in the distinction between PHM, CHM and non-molar hydropic abortions [13,15,16,39-41]. Before the introduction of these ancillary techniques, some complete hydatidiform moles could have incorrectly been classified as PHM or non-molar abortions, explaining the wide variation and changes in reported ratios between complete and partial hydatidiform moles [15,32,40].

HUMAN CHORIONIC GONADOTROPIN

The glycoprotein hCG is a sensitive marker for monitoring trophoblastic activity in pregnancy and gestational trophoblastic disease (GTD) [2,42]. To enable early detection of those patients requiring further treatment, all patients with hydatidiform mole should be subjected to continued hCG surveillance [17]. hCG is a heterogeneous molecule, produced by trophoblastic tissue during pregnancy [42]. Beside intact hCG, consisting of an α- and β-subunit, other forms of hCG are present in serum such as hyperglycosylated hCG, nicked hCG, free β-subunit, free α-subunit, nicked free β-subunit, and β-core fragments [43]. The production of subunits is under stringent physiological control in normal pregnancy and likely differs in pathological events such as a hydatidiform mole

[43]. It is therefore essential to use an hCG assay that detects different forms of hCG [44]. Although sandwich-type assays can be directed against a variety of epitopes on the hCG molecule, the wide range of epitope combinations on the hCG molecule may result in different specificity for hCG and its subunits with different hCG assays in use [45,46].

In the Netherlands, an in-house developed radioimmunoassay (RIA), based on polyclonal antibodies raised in rabbits is used for all measurements in sera sent to the Dutch Central Registry for Hydatidiform Moles since 1977 [47]. The assay is designed to detect all native hCG with emphasis on free β-subunit. A highly purified hCG β-subunit preparation labeled with Iodine-125 is used as a tracer. The assay is calibrated with the third International Standard (IS) for Chorionic Gonadotrophin (WHO, 75-537). The cutoff value for normal hCG levels is established at 2 ng/mL, representing the cutoff serum concentration at 95% specificity as found in postmenopausal women [48].

HCG REGRESSION

Several authors have described serum hCG regression curves, based on patients with uneventful serum hCG regression after evacuation of a hydatidiform mole [47,49,50]. These regression curves can be used as a reference while monitoring patients after molar evacuation. Some authors found that an hCG regression curve, based on patients with uneventful hCG regression allowed earlier detection of patients developing post-molar GTN than the FIGO 2000 guidelines [47,49]. In the Netherlands, the application of such a normogram has resulted in an additional criterion for post-molar GTN. The Dutch criteria for post-molar GTN include: 1) a plateau in serum hCG for 4 consecutive measurements over a period of 3 weeks, 2) or a rise in serum hCG for 3 consecutive measurements over a period of 2 weeks, 3) and at least one of the values should exceed the 95th percentile of an hCG normogram of uneventful hCG decline as constructed by Yedema et al. [47]. The reason for this additional criterion was the observation that 15% of patients with an initial plateau or rise of serum hCG, demonstrated a spontaneous normalization [47]. The normogram as presented by Yedema in 1993, has been widely used as a reference for both complete and partial hydatidiform moles and allows close monitoring of patients, while facilitating identification of those developing GTN [47]. The normogram was however solely based on uneventful regres-

sion after complete hydatidiform moles and similar hCG regression in patients with partial hydatidiform moles was only presumed. Considering the distinctive features in terms of genetics, histology and clinical presentation between complete and partial hydatidiform moles, this presumption can be questioned. Moreover, the normogram was based on patients with uneventful hCG regression between 1977 and 1989, when hydatidiform moles typically presented in the second trimester with classic clinical signs of vaginal bleeding, uterine enlargement and hyperemesis [47,51-53]. Since the early 1990s, ultrasonography was widely introduced as part of routine first-trimester clinical management. Consequently, hydatidiform mole patients are generally diagnosed at an earlier gestational age, before classical symptoms have developed. As a result, lower hCG levels at time of evacuation and earlier hCG normalization are expected today and a shift in hCG regression may be anticipated [52,54,55].

PREDICTION OF GTN

A number of studies focused on the challenging prospect of predicting post-molar GTN. A tool that enables individualized and timely prediction of post-molar GTN could be used to either reassure patients at low-risk of post-molar GTN or to identify patients at high-risk of post-molar GTN.

Patients at high-risk of post-molar GTN may benefit from early start of curative chemotherapeutic treatment [56,57]. Some authors explored the predictive value of different biomarkers, including inhibin, progesterone, interleukin-β and CA-125 for post-molar GTN [58-63]. Although these biomarkers are used for diagnosis and follow-up of other gynecologic malignancies, none of these markers can reliably predict which patients will undergo a malignant change to post-molar GTN. Most research on predicting post-molar GTN has focused on serum hCG and different hCG-subunits [43,64,65]. Van Trommel et al. has shown that hCGα, hCGβ and hCG+hCGβ concentrations are significantly elevated in patients who will develop post-molar GTN when compared to patients with spontaneous regression after molar evacuation. Others have discussed the potential to predict post-molar GTN with the use of other markers such as hyperglycosylated hCG or the calculation of hCG ratios [43,65-69]. Although these studies underline the relevance of serum hCG in post-molar GTN, none of these findings resulted in a sufficiently accurate prediction tool to be used in daily practice and diagnosis of

post-molar GTN is still based on the criteria as defined by the International Federation of Obstetrics and Gynecology (FIGO) [70].

GESTATIONAL TROPHOBLASTIC NEOPLASIA

Although gestational trophoblastic neoplasia generally occurs after evacuation of a hydatidiform mole, GTN may follow after any kind of gestation including abortion and normal pregnancy [71]. It is usually unknown whether GTN represents choriocarcinoma or invasive mole, since histological confirmation is not essential for classification and subsequent choice of therapy [1,72]. Biopsy of lesions suggestive of GTN is best avoided since massive bleeding may occur [1]. When diagnosis of GTN is established, usually through hCG follow-up, a metastatic workup with an evaluation for the presence of risk factors should be performed [22,73]. Chest X-ray is essential, as pulmonary metastases are most common [17]. If lesions are visible on chest X-ray, MRI of the brain and CT thorax/abdomen are indicated to evaluate the presence of widespread disease, which would significantly alter treatment and prognosis [1,21]. PSTT and ETT should be suspected in case of poor response to chemotherapeutic treatment, especially when the disease has developed after a non-molar gestation and hCG concentrations are low [21,74].

SECOND CURETTAGE

In some studies, a second uterine curettage has been recommended for patients with GTN [75], most clinicians are however reluctant to recommend routine curettage for patients with post-molar GTN [76,77]. Generally, the procedure is only used to remove residual intra-uterine disease or to control persistent vaginal hemorrhage [77,78]. The possible benefit of a second curettage has been investigated in several retrospective studies. Reported cure rates were inconsistent, with success rates ranging from 9% to 60% and major complications such as uterine perforation or hemorrhage occurring as often as 8% [75-77]. Recently, Osborne et al. [78] conducted a prospective multicenter trial to better define the efficacy and safety of a second curettage for non-metastatic post-molar GTN. They reported cure rates in 40% of patients with non-metastatic GTN, whereas major complications occurred in 5% of cases [78]. Although these results

seem promising, reported success rates may depend on the selection of patients, GTN definition and technique used for second uterine curettage. Further research, exploring baseline patient characteristics and histology is needed to further evaluate the added value of a second curettage in this patient group.

CLASSIFICATION OF GTN

When diagnosis of GTN is made and metastatic workup is performed, patients are classified to low-risk or high-risk disease with the use of a prognostic scoring system[79]. Patients with PSTT and ETT should be classified separately as clinical presentation and management differs [80-83]. Historically, several important predictors of unfavorable prognosis such as serum hCG levels, age, antecedent pregnancy and site of metastases have been proposed for patients with GTN [84,85]. In 1976, Bagshawe [84] suggested a weighted prognostic scoring system, considering various prognostic factors. It provided the basis for several other classification systems, used to predict the potential for resistance to first-line chemotherapy [70,84,86]. The use of these various clinical classification systems has proven to be effective, but with a variety of classification systems worldwide, meaningful comparison of clinical results was still compromised [70,84,87,88]. Therefore, with the effort of a number of international societies including the International Society for the Study of Trophoblastic Diseases (ISSTD) and the International Gynecologic Cancer Society (IGCS) the FIGO 2000 classification system (Table 1) was developed combining both anatomic and clinical factors [70,79,87]. Patients with a score of 0-6 are considered low-risk, while patients with a score of 7 or higher are considered high-risk [70,79,87]. The worldwide introduction of the FIGO 2000 has provided an opportunity to reach agreement on classification and subsequent treatment for patients with GTN. The system is however quite elaborate with an extensive set of risk factors, where many relate to tumor bulk and are likely interrelated [85,89-91]. In the Netherlands, a classification system as proposed by the Dutch Working Party on trophoblastic disease is still in use (Table 2) [86,92]. The Dutch system is still employed today due to the use of a small set of easily retrievable factors and the assumption that serious prognostic factors such as liver involvement and antecedent pregnancy are taken as absolute criteria for high-risk treatment [92]. The use of a separate risk classification system however excludes the Netherlands from international comparison of research results.

Table 1. FIGO 2000 Classification system for GTN

Score	0	1	2	4
Age (years)	<40	≥40	-	-
Antecedent pregnancy	Mole	Abortion	Term	-
Interval from index pregnancy (months)	<4	4-6	7-12	≥13
Pre-treatment serum hCG (IU/L)	$<10^3$	$<10^4$	$<10^5$	$\geq 10^5$
Largest tumor size (cm)	<3	3-4	≥5	-
Site of metastases	Lung	Spleen, kidney	Gastro-intestinal	Liver, brain
Number of metastases	-	1-4	5-8	>8
Previous failed chemotherapy	-	-	Single drug	Multi drug

A total score of 0-6 = low-risk; score ≥ 7 = high-risk

Table 2. Dutch classification system for GTN[a]

Risk group	Criteria
Low-risk	1. Antecedent pregnancy was a mole or miscarriage
	2. Metastases confined to vagina or lungs
	3. No previous failed chemotherapy
	4. Interval from end of index pregnancy to treatment does not exceed 12 months
High-risk	1. Antecedent pregnancy was a term pregnancy
	2. Metastases in more than one site (outside the uterus)
	3. Metastases in one or more of the following organs: brain, liver, spleen, kidney, gastrointestinal tract
	4. Failure of previous chemotherapy
	5. Interval from end of index pregnancy to treatment exceeds 12 months

[a] When one of the high-risk criteria is present, patients will be classified as high-risk and treated accordingly

MANAGEMENT OF GTN

About 90-95% of patients who develop GTN are at low-risk of developing resistance to single-agent chemotherapy [93]. These patients are usually treated with methotrexate combined with folinic acid and actinomycin D as an acceptable alternative in case of toxicity or resistance [3,21,74]. Patients failing first-line therapy, usually because of resistance, can be salvaged with second and occasionally third-line chemotherapy, resulting in an overall survival approaching 100% [94,95]. Patients with high-risk classification are very unlikely to be cured with single-agent chemotherapy and should therefore be treated with a multi-agent chemotherapy regimen [1,83]. Overall reported five-year survival for high-risk patients varies between 75% and 90% [96-99]. Fatal outcome is predominantly seen in patients with widespread metastatic disease, especially when

liver and/or brain are involved [100-102]. Today, the most widely accepted treatment for high-risk GTN involves the EMA/CO regimen, consisting of etoposide, methotrexate combined with folinic acid and actinomycin D alternating weekly with cyclophosphamide and vincristine [96-99,103-105].

As a result of the improvements in chemotherapeutic treatment, a less important role remains for surgical procedures. Hysterectomy remains the recommended treatment for PSTT as these tumors appear fairly chemo-resistant with a propensity for lymphatic spread [17,80-82]. In perimenopausal patients with GTN, primary hysterectomy can be considered if fertility preservation is not desired [106,107], whereas secondary hysterectomy and metastasectomy (ie pulmonary resection, craniotomy, liver lobe resection) may be essential in chemo-resistant disease [1,102,106,108] or vital hemorrhage [83,107]. Although several authors have assessed the role of surgical procedures in GTN, studies were generally small-sized and the possible benefit of these procedures in daily practice is still unclear [106-109].

OUTLINE OF THE THESIS

This thesis focuses on the detection and classification of GTN, in order to optimize management of patients with this rare but potentially life-threatening condition. In **chapter 2** the trends in incidence of gestational trophoblastic disease in the Netherlands are determined with the use of population-based data. For monitoring trophoblastic activity in both patients with GTD and normal pregnancy, the glycoprotein hormone human chorionic gonatotropin, produced by trophoblastic tissue, is a sensitive marker. So far, Dutch clinicians relied on a normogram as presented by Yedema in 1993, to allow close monitoring of all patients with GTD. The normogram was however solely based on uneventful regression after complete hydatidiform moles and similar behavior of hCG regression in partial hydatidiform moles was only presumed. In addition, due to earlier diagnosis with the introduction of first trimester ultrasonography, lower hCG levels at time of evacuation and earlier hCG normalization are expected. **Chapter 3** describes a new serum hCG normogram for both uneventful complete and partial hydatidiform moles, facilitating follow-up and diagnosis of those patients developing post-molar GTN. **Chapter 4** presents a simple and reliable prediction tool to estimate the individualized risk of post-molar GTN, based on a single

serum hCG measurement taken after evacuation. This tool can be used by clinicians to either reassure patients at low-risk of post-molar GTN or to identify high-risk patients. Early start of chemotherapeutic treatment may be considered for patients at high-risk of post-molar GTN, especially when compliance to follow-up is poor. In general, the treatment of choice in both low-risk and high-risk patients with GTN involves chemotherapy. In selected cases, a hysterectomy may be an effective means to either reduce or eliminate tumor bulk. In **chapter 5** the possible benefits of hysterectomy on treatment duration and treatment toxicity are explored for patients with GTN. Once the decision has been made that chemotherapy is needed, use of a prognostic classification system is an effective means to stratify patients with GTN to single or multi-agent chemotherapy. To validate and improve management of GTN worldwide, it is essential to reach consensus on the used classification system and subsequent treatment protocols. **Chapter 6** presents a comparison between the Dutch classification system and the internationally used FIGO 2000 for patients with GTN. Use of the FIGO 2000 in the Netherlands would enhance comparison with international research results. The system is however quite elaborate with an extensive set of risk factors, where many relate to tumor bulk and interrelation may be presumed. In **chapter 7**, we re-evaluate all prognostic factors involved in the FIGO 2000 classification system and examine whether simplification of this system is feasible.

REFERENCES

1. Seckl MJ, Sebire NJ, Fisher RA, et al. Gestational trophoblastic disease: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24 Suppl 6**: vi39-50.
2. Lurain JR. Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. *Am J Obstet Gynecol* 2010; **203**(6): 531-9.
3. Froeling FE, Seckl MJ. Gestational trophoblastic tumours: an update for 2014. *Curr Oncol Rep* 2014; **16**(11): 408.
4. Wells M. The pathology of gestational trophoblastic disease: recent advances. *Pathology* 2007; **39**(1): 88-96.
5. Hui P, Martel M, Parkash V. Gestational trophoblastic diseases: recent advances in histopathologic diagnosis and related genetic aspects. *Adv Anat Pathol* 2005; **12**(3): 116-25.
6. Cheung AN. Pathology of gestational trophoblastic diseases. *Best Pract Res Clin Obstet Gynaecol* 2003; **17**(6): 849-68.
7. Bentley RC. Pathology of gestational trophoblastic disease. *Clin Obstet Gynecol* 2003; **46**(3): 513-22.
8. Deavers MT, Kalhor N, Silva EG. Diagnostic problems with trophoblastic lesions. *Arch Pathol Lab Med* 2008; **132**(2): 168-74.
9. Shih IM, Kurman RJ. The pathology of intermediate trophoblastic tumors and tumor-like lesions. *Int J Gynecol Pathol* 2001; **20**(1): 31-47.
10. Berkowitz RS, Goldstein DP. Clinical practice. Molar pregnancy. *N Engl J Med* 2009; **360**(16): 1639-45.
11. Hancock BW, Tidy JA. Current management of molar pregnancy. *J Reprod Med* 2002; **47**(5): 347-54.
12. Mangili G, Garavaglia E, Cavoretto P, Gentile C, Scarfone G, Rabaiotti E. Clinical presentation of hydatidiform mole in northern Italy: has it changed in the last 20 years? *Am J Obstet Gynecol* 2008; **198**(3): 302 e1-4.
13. Ronnett BM, DeScipio C, Murphy KM. Hydatidiform moles: ancillary techniques to refine diagnosis. *Int J Gynecol Pathol* 2011; **30**(2): 101-16.
14. Buza N, Hui P. Immunohistochemistry and other ancillary techniques in the diagnosis of gestational trophoblastic diseases. *Semin Diagn Pathol* 2014; **31**(3): 223-32.
15. Chen KH, Hsu SC, Chen HY, Ng KF, Chen TC. Utility of fluorescence in situ hybridization for ploidy and p57 immunostaining in discriminating hydatidiform moles. *Biochem Biophys Res Commun* 2014; **446**(2): 555-60.
16. Castrillon DH, Sun D, Weremowicz S, Fisher RA, Crum CP, Genest DR. Discrimination of complete hydatidiform mole from its mimics by immunohistochemistry of the paternally imprinted gene product p57KIP2. *Am J Surg Pathol* 2001; **25**(10): 1225-30.
17. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet* 2010; **376**(9742): 717-29.
18. Feltmate CM, Batorfi J, Fulop V, Goldstein DP, Doszpod J, Berkowitz RS. Human chorionic gonadotropin follow-up in patients with molar pregnancy: a time for reevaluation. *Obstet Gynecol* 2003; **101**(4): 732-6.
19. Hancock BW, Nazir K, Everard JE. Persistent gestational trophoblastic neoplasia after partial hydatidiform mole incidence and outcome. *J Reprod Med* 2006; **51**(10): 764-6.

20. Benedet JL, Bender H, Jones H, 3rd, Ngan HY, Pecorelli S. FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. FIGO Committee on Gynecologic Oncology. *Int J Gynaecol Obstet* 2000; **70**(2): 209-62.
21. Mangili G, Lorusso D, Brown J, et al. Trophoblastic disease review for diagnosis and management: a joint report from the International Society for the Study of Trophoblastic Disease, European Organisation for the Treatment of Trophoblastic Disease, and the Gynecologic Cancer InterGroup. *Int J Gynecol Cancer* 2014; **24**(9 Suppl 3): S109-16.
22. Ngan HY, Seckl MJ, Berkowitz RS, et al. Update on the diagnosis and management of gestational trophoblastic disease. *Int J Gynaecol Obstet* 2015; **131 Suppl 2**: S123-6.
23. Golfier F. Persistently raised and falling hCG at 6 months no longer a criterion for post molar gestational trophoblastic neoplasia? *BJOG* 2016; **123**(7): 1182.
24. Palmer JR. Advances in the epidemiology of gestational trophoblastic disease. *J Reprod Med* 1994; **39**(3): 155-62.
25. Lybol C, Thomas CM, Bulten J, van Dijck JA, Sweep FC, Massuger LF. Increase in the incidence of gestational trophoblastic disease in The Netherlands. *Gynecol Oncol* 2011; **121**(2): 334-8.
26. Matsui H, Iitsuka Y, Yamazawa K, Tanaka N, Seki K, Sekiya S. Changes in the incidence of molar pregnancies. A population-based study in Chiba Prefecture and Japan between 1974 and 2000. *Hum Reprod* 2003; **18**(1): 172-5.
27. Smith HO, Hilgers RD, Bedrick EJ, et al. Ethnic differences at risk for gestational trophoblastic disease in New Mexico: A 25-year population-based study. *Am J Obstet Gynecol* 2003; **188**(2): 357-66.
28. Salehi S, Eloranta S, Johansson AL, Bergstrom M, Lambe M. Reporting and incidence trends of hydatidiform mole in Sweden 1973-2004. *Acta Oncol* 2011; **50**(3): 367-72.
29. Kim SJ, Lee C, Kwon SY, Na YJ, Oh YK, Kim CJ. Studying changes in the incidence, diagnosis and management of GTD: the South Korean model. *J Reprod Med* 2004; **49**(8): 643-54.
30. Shi YF, Li JQ, Zheng W, et al. [Survey of gestational trophoblastic disease incidence among 3.6 million pregnancies in China]. *Zhonghua fu chan ke za zhi* 2005; **40**(2): 76-8.
31. Altieri A, Franceschi S, Ferlay J, Smith J, La Vecchia C. Epidemiology and aetiology of gestational trophoblastic diseases. *Lancet Oncol* 2003; **4**(11): 670-8.
32. Smith HO. Gestational trophoblastic disease epidemiology and trends. *Clin Obstet Gynecol* 2003; **46**(3): 541-56.
33. Bracken MB. Incidence and aetiology of hydatidiform mole: an epidemiological review. *Br J Obstet Gynaecol* 1987; **94**(12): 1123-35.
34. Semer DA, Macfee MS. Gestational trophoblastic disease: epidemiology. *Semin Oncol* 1995; **22**(2): 109-12.
35. Gestational trophoblastic diseases. Report of a WHO Scientific Group. *World Health Organ Tech Rep Ser* 1983; **692**: 7-81.
36. Fukunaga M, Katabuchi H, Nagasaka T, Mikami Y, Minamiguchi S, Lage JM. Interobserver and intraobserver variability in the diagnosis of hydatidiform mole. *Am J Surg Pathol* 2005; **29**(7): 942-7.
37. Howat AJ, Beck S, Fox H, et al. Can histopathologists reliably diagnose molar pregnancy? *J Clin Pathol* 1993; **46**(7): 599-602.
38. Sebire NJ, Fisher RA, Rees HC. Histopathological diagnosis of partial and complete hydatidiform mole in the first trimester of pregnancy. *Pediatr Dev Pathol* 2003; **6**(1): 69-77.

39. Grinschgl I, Mannweiler S, Holzapfel-Bauer M, Pferschy U, Hoefler G, Guertl B. The role of morphology in combination with ploidy analysis in characterizing early gestational abortion. *Virchows Arch* 2013; **462**(2): 175-82.
40. Jeffers MD, O'Dwyer P, Curran B, Leader M, Gillan JE. Partial hydatidiform mole: a common but underdiagnosed condition. A 3-year retrospective clinicopathological and DNA flow cytometric analysis. *Int J Gynecol Pathol* 1993; **12**(4): 315-23.
41. Fukunaga M. Early partial hydatidiform mole: prevalence, histopathology, DNA ploidy, and persistence rate. *Virchows Arch* 2000; **437**(2): 180-4.
42. Thomas CM, Reijnders FJ, Segers MF, Doesburg WH, Rolland R. Human choriogonadotropin (hCG): comparisons between determinations of intact hCG, free hCG beta-subunit, and "total" hCG + beta in serum during the first half of high-risk pregnancy. *Clin Chem* 1990; **36**(4): 651-5.
43. van Trommel NE, Sweep FC, Schijf CP, Massuger LF, Thomas CM. Diagnosis of hydatidiform mole and persistent trophoblastic disease: diagnostic accuracy of total human chorionic gonadotropin (hCG), free hCG {alpha}- and {beta}-subunits, and their ratios. *Eur J Endocrinol* 2005; **153**(4): 565-75.
44. Birken S, Krichevsky A, O'Connor J, et al. Development and characterization of antibodies to a nicked and hyperglycosylated form of hCG from a choriocarcinoma patient: generation of antibodies that differentiate between pregnancy hCG and choriocarcinoma hCG. *Endocrine* 1999; **10**(2): 137-44.
45. Cole LA. Phantom hCG and phantom choriocarcinoma. *Gynecol Oncol* 1998; **71**(2): 325-9.
46. Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women with false-positive human chorionic gonadotropin concentrations. *Lancet* 2000; **355**(9205): 712-5.
47. Yedema KA, Verheijen RH, Kenemans P, et al. Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. *Am J Obstet Gynecol* 1993; **168**(3 Pt 1): 787-92.
48. Thomas CM, Segers MF, Houx PC. Comparison of the analytical characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. *Ann Clin Biochem* 1985; **22 (Pt 3)**: 236-46.
49. Shigematsu T, Kamura T, Saito T, Kaku T, Nakano H, Kinugawa N. Identification of persistent trophoblastic diseases based on a human chorionic gonadotropin regression curve by means of a stepwise piecewise linear regression analysis after the evacuation of uneventful moles. *Gynecol Oncol* 1998; **71**(3): 376-80.
50. Behtash N, Ghaemmaghami F, Honar H, et al. Is normal beta-hCG regression curve helpful in the diagnosis of persistent trophoblastic disease? *Int J Gynecol Cancer* 2004; **14**(5): 980-3.
51. Curry SL, Hammond CB, Tyrey L, Creasman WT, Parker RT. Hydatidiform mole: diagnosis, management, and long-term followup of 347 patients. *Obstet Gynecol* 1975; **45**(1): 1-8.
52. Soto-Wright V, Bernstein M, Goldstein DP, Berkowitz RS. The changing clinical presentation of complete molar pregnancy. *Obstet Gynecol* 1995; **86**(5): 775-9.
53. Hou JL, Wan XR, Xiang Y, Qi QW, Yang XY. Changes of clinical features in hydatidiform mole: analysis of 113 cases. *J Reprod Med* 2008; **53**(8): 629-33.
54. Jauniaux E, Nicolaides KH. Early ultrasound diagnosis and follow-up of molar pregnancies. *Ultrasound Obstet Gynecol* 1997; **9**(1): 17-21.

55. Kerkmeijer LG, Massuger LF, Ten Kate-Booij MJ, Sweep FC, Thomas CM. Earlier diagnosis and serum human chorionic gonadotropin regression in complete hydatidiform moles. *Obstet Gynecol* 2009; **113**(2 Pt 1): 326-31.
56. Cagayan MS. Changing trends in the management of gestational trophoblastic diseases in the Philippines. *J Reprod Med* 2010; **55**(5-6): 267-72.
57. Park TK, Kim SN, Lee SK. Analysis of risk factors for postmolar trophoblastic disease: categorization of risk factors and effect of prophylactic chemotherapy. *Yonsei Med J* 1996; **37**(6): 412-9.
58. Kohorn EI. Measurement of CA-125 in trophoblastic disease. *Gynecol Oncol* 2000; **78**(1): 39-42.
59. Hegab HM, Schindler AE, Rizk M, Ramadan M. Evaluation of tumour markers in molar pregnancy. *Arch Gynecol Obstet* 2003; **268**(3): 151-4.
60. Mungan T, Kuscu E, Ugur M, Dabakoglu T, Senses E, Cobanoglu O. Screening of persistent trophoblastic disease with various serum markers. *Eur J Gynaecol Oncol* 1998; **19**(5): 495-7.
61. Hegab HM, Schindler AE. The prognostic value of serum inhibin, 17 beta-estradiol and progesterone in cases of hydatidiform mole. *Gynecol Endocrinol* 2004; **18**(2): 107-13.
62. Seki K, Matsui H, Sekiya S. Advances in the clinical laboratory detection of gestational trophoblastic disease. *Clin Chim Acta* 2004; **349**(1-2): 1-13.
63. Li RH, Yu MM, Cheung AN, Wong YF. Expression of leptin and leptin receptors in gestational trophoblastic diseases. *Gynecol Oncol* 2004; **95**(2): 299-306.
64. Lybol C, Sweep FC, Ottevanger PB, Massuger LF, Thomas CM. Linear regression of postevacuation serum human chorionic gonadotropin concentrations predicts postmolar gestational trophoblastic neoplasia. *Int J Gynecol Cancer* 2013; **23**(6): 1150-6.
65. Stenman UH, Tiitinen A, Alfthan H, Valmu L. The classification, functions and clinical use of different isoforms of HCG. *Hum Reprod Update* 2006; **12**(6): 769-84.
66. van Trommel NE, Ngo Duc H, Massuger LF, et al. Early identification of persistent trophoblastic disease with serum hCG concentration ratios. *Int J Gynecol Cancer* 2008; **18**(2): 318-23.
67. Cole LA, Butler SA, Khanlian SA, et al. Gestational trophoblastic diseases: 2. Hyperglycosylated hCG as a reliable marker of active neoplasia. *Gynecol Oncol* 2006; **102**(2): 151-9.
68. Duc HN, van Trommel NE, Sweep FC, Massuger LF, Thomas CM. Clinical utility of hyperglycosylated hCG in serum taken before hydatidiform mole evacuation to predict persistent trophoblastic disease. *Int J Biol Markers* 2006; **21**(1): 45-9.
69. Kim BW, Cho H, Kim H, et al. Human chorionic gonadotrophin regression rate as a predictive factor of postmolar gestational trophoblastic neoplasm in high-risk hydatidiform mole: a case-control study. *Eur J Obstet Gynecol Reprod Biol* 2012; **160**(1): 100-5.
70. Ngan HY, Bender H, Benedet JL, et al. Gestational trophoblastic neoplasia, FIGO 2000 staging and classification. *Int J Gynaecol Obstet* 2003; **83 Suppl 1**: 175-7.
71. Beller U, Benedet JL, Creasman WT, et al. Carcinoma of the vagina. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int J Gynaecol Obstet* 2006; **95 Suppl 1**: S29-42.
72. Ngan HY, Kohorn EI, Cole LA, et al. Trophoblastic disease. *Int J Gynaecol Obstet* 2012; **119 Suppl 2**: S130-6.
73. Berkowitz RS, Goldstein DP. Current management of gestational trophoblastic diseases. *Gynecol Oncol* 2009; **112**(3): 654-62.

74. Brown J, Naumann RW, Seckl MJ, Schink J. 15years of progress in gestational trophoblastic disease: Scoring, standardization, and salvage. *Gynecol Oncol* 2016.
75. Pezeshki M, Hancock BW, Silcocks P, et al. The role of repeat uterine evacuation in the management of persistent gestational trophoblastic disease. *Gynecol Oncol* 2004; **95**(3): 423-9.
76. Schlaerth JB, Morrow CP, Rodriguez M. Diagnostic and therapeutic curettage in gestational trophoblastic disease. *Am J Obstet Gynecol* 1990; **162**(6): 1465-70; discussion 70-1.
77. van Trommel NE, Thomas CM, Massuger LF, Sweep FC. Second curettage in persistent trophoblastic disease (PTD): the need for univocal definition of PTD. *Gynecol Oncol* 2005; **99**(1): 250-1; author reply 1.
78. Osborne RJ, Filiaci VL, Schink JC, et al. Second Curettage for Low-Risk Nonmetastatic Gestational Trophoblastic Neoplasia. *Obstet Gynecol* 2016; **128**(3): 535-42.
79. Committee FO. FIGO staging for gestational trophoblastic neoplasia 2000. FIGO Oncology Committee. *Int J Gynaecol Obstet* 2002; **77**(3): 285-7.
80. Papadopoulos AJ, Foskett M, Seckl MJ, et al. Twenty-five years' clinical experience with placental site trophoblastic tumors. *J Reprod Med* 2002; **47**(6): 460-4.
81. Hassadia A, Gillespie A, Tidy J, et al. Placental site trophoblastic tumour: clinical features and management. *Gynecol Oncol* 2005; **99**(3): 603-7.
82. Schmid P, Nagai Y, Agarwal R, et al. Prognostic markers and long-term outcome of placental-site trophoblastic tumours: a retrospective observational study. *Lancet* 2009; **374**(9683): 48-55.
83. Lurain JR. Gestational trophoblastic disease II: classification and management of gestational trophoblastic neoplasia. *Am J Obstet Gynecol* 2011; **204**(1): 11-8.
84. Bagshawe KD. Risk and prognostic factors in trophoblastic neoplasia. *Cancer* 1976; **38**(3): 1373-85.
85. Kim SJ, Bae SN, Kim JH, et al. Effects of multiagent chemotherapy and independent risk factors in the treatment of high-risk GTT--25 years experiences of KRI-TRD. *Int J Gynaecol Obstet* 1998; **60 Suppl 1**: S85-96.
86. Hancock BW. Staging and classification of gestational trophoblastic disease. *Best Pract Res Clin Obstet Gynaecol* 2003; **17**(6): 869-83.
87. Kohorn EI, Goldstein DP, Hancock BW, et al. Workshop Report: Combining the staging system of the International Federation of Gynecology and Obstetrics with the scoring system of the World Heath Organization for Trophoblastic Neoplasia. Report of the Working Committee of the International Society for the Study of Trophoblastic Disease and the International Gynecologic Cancer Society. *Int J Gynecol Cancer* 2000; **10**(1): 84-8.
88. Goldstein DP, Zanten-Przybysz IV, Bernstein MR, Berkowitz RS. Revised FIGO staging system for gestational trophoblastic tumors. Recommendations regarding therapy. *J Reprod Med* 1998; **43**(1): 37-43.
89. Soper JT, Evans AC, Conaway MR, Clarke-Pearson DL, Berchuck A, Hammond CB. Evaluation of prognostic factors and staging in gestational trophoblastic tumor. *Obstet Gynecol* 1994; **84**(6): 969-73.
90. Azab MB, Pejovic MH, Theodore C, et al. Prognostic factors in gestational trophoblastic tumors. A multivariate analysis. *Cancer* 1988; **62**(3): 585-92.
91. Nevin J, Silcocks P, Hancock B, Coleman R, Nakielny R, Lorigan P. Guidelines for the stratification of patients recruited to trials of therapy for low-risk gestational trophoblastic tumor. *Gynecol Oncol* 2000; **78**(2): 92-6.

92. Dijkema HE, Aalders JG, de Bruijn HW, Laurini RN, Willemse PH. Risk factors in gestational trophoblastic disease, and consequences for primary treatment. *Eur J Obstet Gynecol Reprod Biol* 1986; **22**(3): 145-52.

93. Sita-Lumsden A, Short D, Lindsay I, et al. Treatment outcomes for 618 women with gestational trophoblastic tumours following a molar pregnancy at the Charing Cross Hospital, 2000-2009. *Br J Cancer* 2012; **107**(11): 1810-4.

94. McNeish IA, Strickland S, Holden L, et al. Low-risk persistent gestational trophoblastic disease: outcome after initial treatment with low-dose methotrexate and folinic acid from 1992 to 2000. *J Clin Oncol* 2002; **20**(7): 1838-44.

95. Chalouhi GE, Golfier F, Soignon P, et al. Methotrexate for 2000 FIGO low-risk gestational trophoblastic neoplasia patients: efficacy and toxicity. *Am J Obstet Gynecol* 2009; **200**(6): 643 e1-6.

96. Turan T, Karacay O, Tulunay G, et al. Results with EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine) chemotherapy in gestational trophoblastic neoplasia. *Int J Gynecol Cancer* 2006; **16**(3): 1432-8.

97. Lu WG, Ye F, Shen YM, et al. EMA-CO chemotherapy for high-risk gestational trophoblastic neoplasia: a clinical analysis of 54 patients. *Int J Gynecol Cancer* 2008; **18**(2): 357-62.

98. Escobar PF, Lurain JR, Singh DK, Bozorgi K, Fishman DA. Treatment of high-risk gestational trophoblastic neoplasia with etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine chemotherapy. *Gynecol Oncol* 2003; **91**(3): 552-7.

99. Lurain JR, Singh DK, Schink JC. Primary treatment of metastatic high-risk gestational trophoblastic neoplasia with EMA-CO chemotherapy. *J Reprod Med* 2006; **51**(10): 767-72.

100. Ahamed E, Short D, North B, Savage PM, Seckl MJ. Survival of women with gestational trophoblastic neoplasia and liver metastases: is it improving? *J Reprod Med* 2012; **57**(5-6): 262-9.

101. Neubauer NL, Latif N, Kalakota K, et al. Brain metastasis in gestational trophoblastic neoplasia: an update. *J Reprod Med* 2012; **57**(7-8): 288-92.

102. Newlands ES, Holden L, Seckl MJ, McNeish I, Strickland S, Rustin GJ. Management of brain metastases in patients with high-risk gestational trophoblastic tumors. *J Reprod Med* 2002; **47**(6): 465-71.

103. Bower M, Newlands ES, Holden L, et al. EMA/CO for high-risk gestational trophoblastic tumors: results from a cohort of 272 patients. *J Clin Oncol* 1997; **15**(7): 2636-43.

104. Matsui H, Suzuka K, Iitsuka Y, Seki K, Sekiya S. Combination chemotherapy with methotrexate, etoposide, and actinomycin D for high-risk gestational trophoblastic tumors. *Gynecol Oncol* 2000; **78**(1): 28-31.

105. Alifrangis C, Agarwal R, Short D, et al. EMA/CO for high-risk gestational trophoblastic neoplasia: good outcomes with induction low-dose etoposide-cisplatin and genetic analysis. *J Clin Oncol* 2013; **31**(2): 280-6.

106. Doll KM, Soper JT. The role of surgery in the management of gestational trophoblastic neoplasia. *Obstet Gynecol Surv* 2013; **68**(7): 533-42.

107. Hanna RK, Soper JT. The role of surgery and radiation therapy in the management of gestational trophoblastic disease. *Oncologist* 2010; **15**(6): 593-600.

108. Doumplis D, Al-Khatib K, Sieunarine K, et al. A review of the management by hysterectomy of 25 cases of gestational trophoblastic tumours from March 1993 to January 2006. *BJOG* 2007; **114**(9): 1168-71.

109. Brewer JI, Smith RT, Pratt GB. Choriocarcinoma. Absolute 5 year survival rates of 122 patients treated by hysterectomy. *Am J Obstet Gynecol* 1963; **85**: 841-3.

Chapter 2

Trends in incidence for gestational trophoblastic disease over the last 20 years in a population-based study

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ABSTRACT

Background: Gestational trophoblastic disease (GTD) represents a heterogeneous group of disorders. Wide variations in incidence rates are reported worldwide, probably explained by a lack of centralized databases and heterogeneity in case definition. Aim of the present study was to determine the trends in incidence of GTD in the last 20 years with the use of population-based data.

Patients and methods: Data on patients with pathologically confirmed diagnosis of GTD between 1994 and 2013 were obtained from PALGA, a nationwide archive containing all pathology reports in the Netherlands.

Results: In the 20-year period 6341 cases were registered with GTD, representing an overall incidence rate of 1.66 per 1000 deliveries per year. An initial rise in incidence rate was seen over the first 10 years (0.075 per year, 95% CI 0.040-0.109), followed by a stabilization from 2004 to 2013 (increase per year 0.011, 95% CI -0.017-0.040). Although partial hydatidiform mole (HM) was more common in earlier years, complete and partial HM reached comparable incidence rates of 0.68 and 0.64 per 1000 deliveries respectively from 2009 onwards. In the last decade, unspecified HM diagnosis declined significantly from 0.14 per 1000 deliveries in 2003 to 0.03 per 1000 deliveries (per year -0.011, CI -0.016---0.06), suggesting improved diagnostic analyses.

Conclusion: After an initial rise in GTD incidence in the Netherlands, rates remained steady from 2004 onwards. As pathological confirmation is currently the norm and advanced pathological techniques are now widely available, true steady incidence rates may have been reached.

INTRODUCTION

Gestational trophoblastic disease (GTD) represents a heterogeneous group of disorders with abnormal proliferation of placental trophoblastic tissue. It encompasses complete and partial hydatidiform mole, invasive mole, choriocarcinoma, placental site trophoblastic tumor, epithelioid trophoblastic tumor, exaggerated placental-site reaction and placental-site nodule.

Our group has previously reported a GTD incidence rate of 1.56 per 1000 deliveries in the Netherlands in the period of 1995 to 2008, with an incidence of HM of 1.34 per 1000 deliveries per year. The overall incidence of GTD was low but showed a significant increase during the aforementioned time span [1]. The incidence rate of HM was broadly comparable with recent findings of population-based studies in Sweden (1.2 per 1000 deliveries), Turkey (1.22 per 1000 deliveries) and the United States (1.19 per 1000 pregnancies) [2-4]. Reported frequencies from Asian countries are highly heterogeneous with rates ranging from 1 to 3 per 1000 pregnancies in Japan, China and Korea to over 10 per 1000 pregnancies in Indonesia and India [2,3,5-8]. Lack of a centralized databases, inability to adequately describe the population at risk and heterogeneity in case definition have probably contributed to the wide variations in incidence rates worldwide. Furthermore, incidence rates may be based on total number of pregnancies, deliveries or live births [9-12].

Although of great clinical relevance, comparing incidence rates with respect to choriocarcinoma and PSTT is even more difficult as these conditions are very rare and pathological confirmation is not always available [8,9,13].

In the Netherlands a national pathology database named PALGA (Pathologisch Anatomisch Landelijk Geautomatiseerd Archief) with nationwide coverage since 1991 holds all records of histopathologic and cytopathologic diagnoses to facilitate research and quality control. This unique nationwide network provides a complete insight in incidence rates in the Netherlands [14].

The present study evaluates trends in incidence for GTD in the Netherlands between 1994 and 2013 using the population-based pathology database PALGA.

Material and methods

Database

The PALGA database is a nationwide archive containing all pathology reports in the Netherlands. Present study identified all cases of GTD recorded in the PALGA database between 1994 and 2013.

Patient selection

A selection of the database was performed for patients diagnosed with GTD on the following search criteria: complete mole (CHM), partial mole (PHM), mole, invasive mole, choriocarcinoma, metastasis choriocarcinoma, intratubular choriocarcinoma, persistent trophoblastic disease (PTD), trophoblastic proliferation, placental site trophoblastic tumor (PSTT), trophoblastic pseudotumor and epithelioid trophoblastic tumor (ETT), placental site nodule and exaggerated placental site reaction, identifying 7530 records. Reports were subsequently reviewed and categorized according to the WHO classification of GTD: complete and partial hydatidiform mole, invasive mole, choriocarcinoma, placental site trophoblastic tumor, epithelioid trophoblastic tumor, exaggerated placental-site reaction and placental-site nodule. Multiple abstracts per patient involving revisions of one specimen were combined, multiple gestational trophoblastic events per patient were however maintained.

Two categories were added: patients with clear diagnoses of HM not otherwise specified and patients where molar pregnancy could not be distinguished from abortion. Inconclusive reports were analyzed and classified by an experienced pathologist, specialized in gynecology. Revisions from abroad (*N*=8) and patients eventually not classified as GTD (*N*=326) were excluded from further analysis. A total of 6341 cases were included in the present study.

Statistical analyses

Incidence rates were calculated per 1000 deliveries for HM, GTD, exaggerated placental site reaction and placental site nodule and per 100,000 deliveries for choriocarcinoma and PSTT annually. Data on the number of deliveries nationwide were obtained from Statistics Netherlands (CBS) [15]. All analyses were carried out using Microsoft Office Excel 2007 and SPSS for Windows (version 20).

RESULTS

Gestational trophoblastic disease

In the 20-year period between 1994 and 2013, a total of 6341 cases were registered with gestational trophoblastic disease in the Netherlands, representing an overall incidence rate of 1.66 per 1000 deliveries per year. Their median age was 31 years (minimum 13, maximum 79). To evaluate potential trends in the annual incidence rates, Figure 1 shows the annual incidence rates in the Netherlands between 1994 and 2013. An initial significant increase is seen over the first 8 years (0.075 per 1000 deliveries per year, 95% CI 0.040-0.109), followed by a stabilized incidence rate from 2002 to 2013 (increase per 1000 deliveries per year 0.011, 95% CI -0.017-0.040). An overview of the average frequency and incidence of individual entities of GTD is shown in Table 1 with moles comprising the majority of cases.

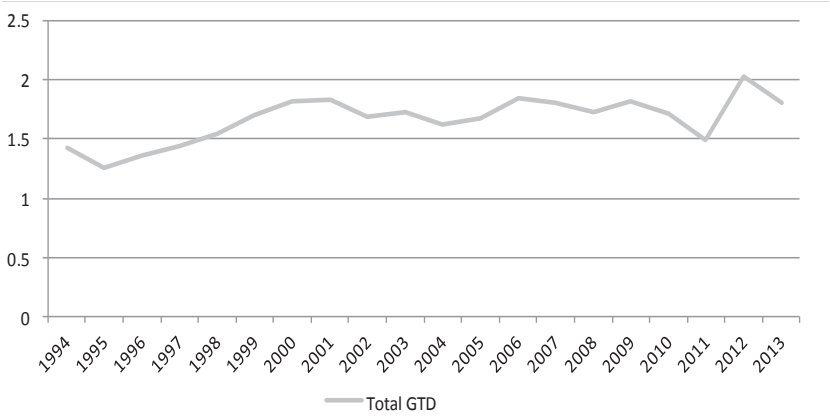

Figure 1. Incidence rate per 1000 deliveries for GTD patients in the Netherlands between 1994 and 2013

Table 1. Average frequency and incidence of individual entities of GTD, 1994-2013

GTD diagnosis	Number	Percentage	Incidence[a]
Complete Hydatidiform Mole	1993	31.4	0.52
Partial Hydatidiform Mole	2548	40.2	0.67
Invasive Mole	18	0.3	0.01
Unspecified Mole	594	9.4	0.16
Abortion or Mole	449	7.1	0.12
Choriocarcinoma	121	1.9	0.03
Placental Site Trophoblastic Tumor	36	0.6	0.01
Epitheloid Trophoblastic Tumor	4	0.1	0.00
Exaggerated Placental Site Reaction	272	4.3	0.07
Placental Site Nodule	306	4.8	0.08
Total	6341	100	1.66

[a] Incidence per 1000 deliveries

Benign trophoblastic lesions

Since 1994 a total of 578 cases with a benign trophoblastic lesion originating from the intermediate trophoblast were recorded in the Netherlands, involving 306 (52.9%) patients with placental site nodule (PSN) and 272 (47.1%) patients with exaggerated placental site (EPS). As shown in Figure 2, a significant rise in incidence for placental site nodules is apparent from 2004 onwards (increase per 1000 deliveries per year

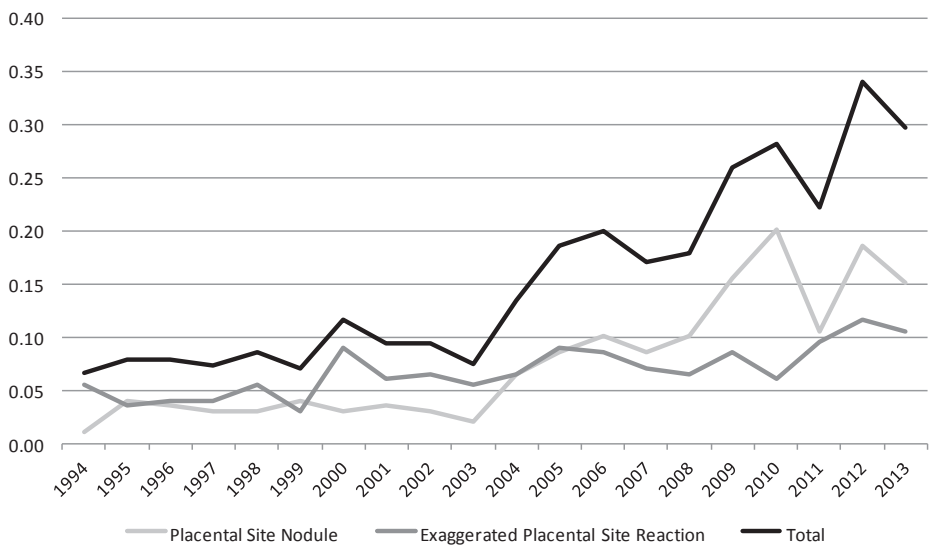

Figure 2. Incidence rates per 1000 deliveries for benign trophoblastic lesions in the Netherlands between 1994 and 2013.

0.012, CI 0.03-0.20), whereas the incidence rise in exaggerated placental site lesions showed a small increase over the years (increase per 1000 deliveries per year 0.003, CI 0.002-0.004). Median age was 33 (minimum 19, maximum 79) and 32 years (minimum 16, maximum 58) for placental site nodule and exaggerated placental site respectively, with 89.0% of patients under 40 years of age. To evaluate whether knowledge of these conditions may be associated with changes in incidence, the number of Pubmed hits for each entity per year was calculated. A very modest number of hits varying from 0 to 7 hits per year however was seen over the years without an apparent increase.

Hydatidiform mole

In Figure 3 the trends in annual incidence rates for HM over the years are shown. Between 1994 and 2013 a total of 5153 cases with HM were registered in the Netherlands resulting in a total incidence of 1.36 moles (complete, partial, unspecified) per 1000 deliveries per year. This comprises 81.0% of all GTD. Comparable with the incidence pattern in total GTD, a significant increase in molar pregnancies per 1000 deliveries per year was apparent between 1994 and 2001 (increase per year 0.068, CI 0.051 – 0.085) followed by an unchanged level since 2002 (increase per year 0.002 , CI -0.023 – 0.027).

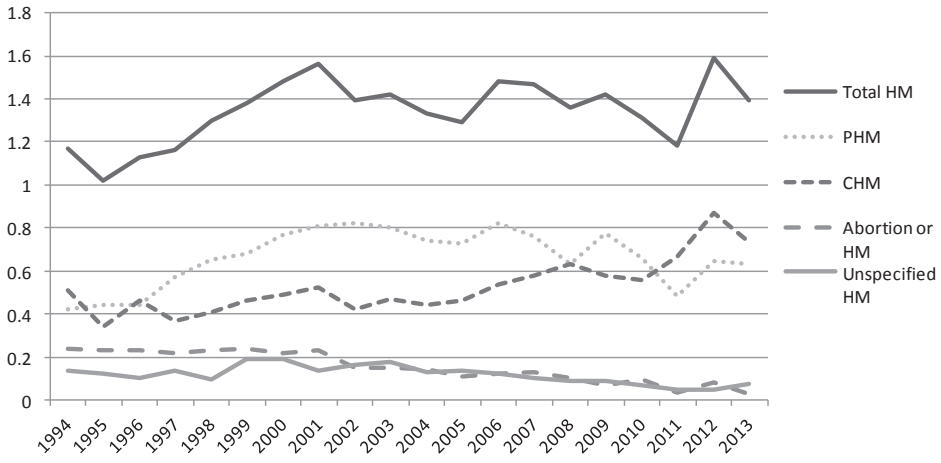

Figure 3. Incidence rates per 1000 deliveries for HM pregnancies in the Netherlands over time (1994-2013)

CHM and PHM represented 31.4% (incidence 0.52 per 1000 deliveries) and 40.2% (incidence 0.67 per 1000 deliveries) of all GTD cases, respectively. A gradual increase in CHM was seen in recent years with incidence rates increasing from 0.46 per 1000 deliveries in 2005 to 0.74 per 1000 deliveries in 2013 (per year 0.036, CI 0.014-0.059). PHM showed a significant increase in incidence from 1994 to 2002 (increase per year 0.058, CI 0.047-0.069), followed by a significant decline from 2003 onwards (per year -0.021, CI -0.037--0.05). The incidence rates for both CHM and PHM have become more comparable in recent years, resulting in a change of CHM to PHM ratio from 0.67 to 1.1. in 2000-2008 and 2009-2013 respectively. The mean maternal age for CHM and PHM was 31.1 and 30.4 years respectively. The distribution of CHM and PHM per age group is shown in Figure 4 with a significant over presentation of CHM in the extreme age groups (*P*<0.001).

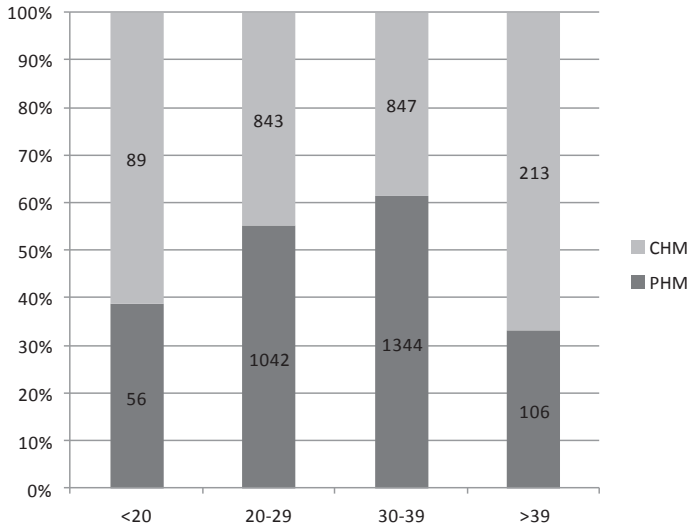

Figure 4. Distribution of CHM and PHM according to maternal age between 1994 and 2013.

Unspecified diagnosis

Patients with a clear diagnosis of HM not otherwise specified and patients with uncertainty concerning discrimination between molar pregnancy and abortion comprised 9.4% and 7.1% of GTD patients. In the last 10 years, the incidence of both these 'unspecified moles' and 'abortion or mole' showed a significant decline as is shown in Figure 3. For unspecified moles the incidence decreased from 0.14 per 1000 deliveries in 2004 to 0.03 per 1000 deliveries in 2013 (per year -0.011, CI -0.016--0.06). For

'abortion or mole' the incidence rate decreased from 0.13 per 1000 deliveries in 2004 to 0.08 per 1000 deliveries in 2013 (per year-0.017, CI -0.032--0.02).

Choriocarcinoma and PSTT

During the 20-year study period 121 cases of choriocarcinoma were reported in the database resulting in an incidence rate of 3 per 100,000 deliveries. Furthermore PSTT and ETT were reported in 36 and 4 cases, resulting in an incidence of 0.9 per 100,000 deliveries and 0.1 per 100,000 deliveries respectively.

DISCUSSION

The present study provides a nation-wide overview of incidence rates for GTD and underlying entities in the Netherlands. Many epidemiologic studies provide incidence rates on GTD by using hospital-based data which inadequately describe the population at risk. In these studies, incidence rates may therefore appear higher. In addition, regional incidence rates of referral treatment centers cannot be merely compared to birth rates in the same region as the region for referral in GTD may be derived from a larger geographic region, underestimating the population at risk.

The incidence rates were reported as number of gestational events in relation to the number of deliveries. Total number of pregnancies would be the most appropriate denominator, including live births, still births, abortions and ectopic pregnancies, however data on the total number of pregnancies are not available. Therefore, as the denominator live births underestimates the population at risk, a small overestimation of the incidence rates observed in the present study is presumed.

The overall incidence rate of HM in the present study was estimated at 1.36 per 1000 live births. These rates are fairly consistent with numbers published in population-based studies from Scandinavia, United Kingdom and the United States States [13,16,17]. It remains unclear whether regional reporting routines, definition criteria, genetic factors or advances in pathologic techniques should be considered.

Our study provides support for an increased relative risk of CHM at both the upper and lower extremes of maternal age, whereas PHM is predominantly seen in the middle

of the reproductive period. An age-associated incidence of HM has previously been described, with a greater extent of risk at older age, whereas the absolute number of pregnancies achieved by older women is greatly diminished [18,19]. The age-associated incidence of CHM and PHM is less clear, although these distinct conditions have different histopathological features and clinical implications. It is hypothesized that abnormal fertilization at the beginning and end of the female reproductive period is involved, although the underlying pathophysiological mechanism predisposing CHM instead of PHM still has to be elucidated [18-21].

Furthermore, an interesting change in ratio of CHM to PHM was seen in the present study. This resulted in comparable incidence rates for CHM and PHM in recent years. In literature a wide range of ratios has been reported ranging from 0.3 to 3.0 [8,22]. Morphologically both CHM and PHM have distinct histopathological features, the subjective nature of the morphological abnormalities however give rise to inter-observer variation in diagnosis [23,24]. In particular when earlier evacuation is performed in the present ultrasound era, classic morphological features will be less apparent [25,26]. The introduction of immune-staining with $p57^{kip2}$, an imprinted marker expressed only by the maternal genome and therefore absent in CHM but present in PHM and the aforementioned ploidy analysis can be helpful in the distinction between PHM, CHM and non molar hydropic abortions [27-29]. Before its introduction CHM could have incorrectly been classified as PHM or non-molar abortions, explaining the wide variation and changes in ratios [8,22,28]. The incidence of both 'unspecified moles' and 'abortion or mole' also showed a decrease over time, further supporting this hypothesis. The ploidy analysis should be performed on selected paraffin embedded slides instead of the complete sample as maternal decidua will obscure results, containing maternal diploid karyotype. Review by a histopathological specialist is therefore recommended.

An accurate incidence rate of choriocarcinoma and PSTT/ETT is less well known. Problems arise as variations in diagnostic criteria and study intervals exist. In the present study an overall incidence rate of 3 choriocarcinoma per 100.000 deliveries was shown (1.9% of all registered GTD cases). PTT and ETT are even rarer, representing 0.6 % and 0.1 % of all registered cases in the Netherlands. GTD however is primarily diagnosed using clinical parameters such as the biochemical marker hCG and radiological findings, therefore histological parameters are not always available. The reported incidence is therefore likely an underestimation of the true incidence rate.

In contrast to HM and choriocarcinoma, benign lesions of the intermediate trophoblast have been recognized only relatively recent. Both PSN and EPS are usually incidental findings in uterine curettages, biopsies or occasionally in hysterectomy specimens, irregular uterine bleeding has however been reported [30 31]. In the present study, both entities typically occurred in the reproductive age groups, with only few patients over 50 years of age. This likely represents persistence of the lesions following an earlier pregnancy. Local recurrence or progression to persistent disease has not been documented, therefore no specific treatment or follow-up is necessary. The relevance of their existence is represented by possible misdiagnosis of PSTT and ETT. Placental site nodules have been described as the benign counterparts of ETT whereas exaggerated placental site lesions show comparison with PSTT [32,33]. To the best of our knowledge, no previous population-based incidence rates on benign intermediate trophoblastic lesions have been reported. The rise in incidence rate for PSN may be explained by raised awareness of the lesions, this is however not supported by a coexisting rise in hits (publications) on pubmed. In difficult cases, Ki-67 immunostaining can be helpful as the benign counterparts are characterized by low proliferation. In cases where uncertainty about the diagnosis still persists, follow-up with measurement of human chorionic gonadotropin concentration in serum should be recommended [30,33,34].

The influence of ethnicity, with Asian population in particular, has previously been suggested as a possible explanation for differences in GTD incidence [3,6]. The aforementioned lack of centralized databases with predominantly hospital-based data however likely plays a large role. Furthermore, in the Dutch population, the rise in proportion of Asians due to immigration has only shown a rise of 0.9% in 20 years, therefore this alone could not clarify the initial rise in incidence as reported.

Part of the initial increase in GTD incidence found in this study may be explained by improvement of the diagnostic analysis. Due to better understanding of this rather rare condition among pathologists a better distinction between hydropic products of conception and molar pregnancies can be made [32]. HM may have been more common than was generally appreciated in previous years [22]. Furthermore immunostaining with $P57^{kip2}$ and ploidy analyses such as flow cytometry and in situ hybridization can be extremely valuable in pathological diagnosis of partial molar pregnancies by demonstration of triploidy [22,26-29,35]. As pathological confirmation is the norm and di-

agnostic pathological techniques are now widely available and recommended in cases of suspected molar gestation, a true steady incidence rate may have been reached. However, with the increasing popularity of medical induction of spontaneous and induced abortions rather than surgical treatment, it may still be difficult to obtain pathological confirmation for some early conception products [8,36].

A limitation of this study is the retrospective design and although inconclusive reports were reviewed by an experienced pathologist who is specialized in gynecology, no pathological review was performed. Also an overview of used diagnostic tests was not always available from the pathology abstracts as registration of their use was not mandatory.

Strengths of the study include the size of the material and the population-based data over an extended time span. Although many countries monitor and report incidence of GTD, not many have been able to report a population-based overview.

In conclusion, the overall incidence of GTD in the Netherlands has been steady over the past decade. Incidence rates for HM and GTD are consistent with rates from previous population-based studies in Western countries. PHM occurs more frequently than was previously assumed. The introduction of $p57^{kip2}$ and ploidy analysis has likely improved the accuracy of the diagnosis and therefore the actual estimates of GTD cases as identified from the Dutch pathological database. Further research on the actual use and contribution of these techniques to diagnosis and follow-up should be implemented, ideally contributing to a standardized and accurate histopathological approach for all cases suspect of GTD.

REFERENCES

1. Lybol C, Thomas CM, Bulten J, van Dijck JA, Sweep FC, Massuger LF. Increase in the incidence of gestational trophoblastic disease in The Netherlands. *Gynecol Oncol* 2011; **121**(2): 334-8.
2. Matsui H, Iitsuka Y, Yamazawa K, Tanaka N, Seki K, Sekiya S. Changes in the incidence of molar pregnancies. A population-based study in Chiba Prefecture and Japan between 1974 and 2000. *Hum Reprod* 2003; **18**(1): 172-5.
3. Smith HO, Hilgers RD, Bedrick EJ, et al. Ethnic differences at risk for gestational trophoblastic disease in New Mexico: A 25-year population-based study. *Am J Obstet Gynecol* 2003; **188**(2): 357-66.
4. Salehi S, Eloranta S, Johansson AL, Bergstrom M, Lambe M. Reporting and incidence trends of hydatidiform mole in Sweden 1973-2004. *Acta Oncol* 2011; **50**(3): 367-72.
5. Kim SJ, Lee C, Kwon SY, Na YJ, Oh YK, Kim CJ. Studying changes in the incidence, diagnosis and management of GTD: the South Korean model. *J Reprod Med* 2004; **49**(8): 643-54.
6. Shi YF, Li JQ, Zheng W, et al. [Survey of gestational trophoblastic disease incidence among 3.6 million pregnancies in China]. *Zhonghua fu chan ke za zhi* 2005; **40**(2): 76-8.
7. Altieri A, Franceschi S, Ferlay J, Smith J, La Vecchia C. Epidemiology and aetiology of gestational trophoblastic diseases. *Lancet Oncol* 2003; **4**(11): 670-8.
8. Smith HO. Gestational trophoblastic disease epidemiology and trends. *Clin Obstet Gynecol* 2003; **46**(3): 541-56.
9. Lurain JR. Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. *Am J Obstet Gynecol* 2010; **203**(6): 531-9.
10. Bracken MB. Incidence and aetiology of hydatidiform mole: an epidemiological review. *Br J Obstet Gynaecol* 1987; **94**(12): 1123-35.
11. Semer DA, Macfee MS. Gestational trophoblastic disease: epidemiology. *Semin Oncol* 1995; **22**(2): 109-12.
12. Gestational trophoblastic diseases. Report of a WHO Scientific Group. *World Health Organ Tech Rep Ser* 1983; **692**: 7-81.
13. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet* 2010; **376**(9742): 717-29.
14. Casparie M, Tiebosch AT, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol* 2007; **29**(1): 19-24.
15. Database of Statistics Netherlands. http://www.cbs.nl.
16. Flam F, Lundstrom-Lindstedt V, Rutqvist LE. Incidence of gestational trophoblastic disease in Stockholm County, 1975-1988. *Eur J Epidemiol* 1992; **8**(2): 173-7.
17. Olsen JH, Mellemkjaer L, Gridley G, Brinton L, Johansen C, Kjaer SK. Molar pregnancy and risk for cancer in women and their male partners. *Am J Obstet Gynecol* 1999; **181**(3): 630-4.
18. Sebire NJ, Foskett M, Fisher RA, Rees H, Seckl M, Newlands E. Risk of partial and complete hydatidiform molar pregnancy in relation to maternal age. *BJOG* 2002; **109**(1): 99-102.
19. Altman AD, Bentley B, Murray S, Bentley JR. Maternal age-related rates of gestational trophoblastic disease. *Gynecol Oncol* 2008; **112**(2 Pt 1): 244-50.

20. Erturk M, Cakmak HA, Surgit O, et al. The predictive value of elevated neutrophil to lymphocyte ratio for long-term cardiovascular mortality in peripheral arterial occlusive disease. *J Cardiol* 2014; **64**(5): 371-6.
21. Bagshawe KD, Dent J, Webb J. Hydatidiform mole in England and Wales 1973-83. *Lancet* 1986; **2**(8508): 673-7.
22. Jeffers MD, O'Dwyer P, Curran B, Leader M, Gillan JE. Partial hydatidiform mole: a common but underdiagnosed condition. A 3-year retrospective clinicopathological and DNA flow cytometric analysis. *Int J Gynecol Pathol* 1993; **12**(4): 315-23.
23. Fukunaga M, Katabuchi H, Nagasaka T, Mikami Y, Minamiguchi S, Lage JM. Interobserver and intraobserver variability in the diagnosis of hydatidiform mole. *Am J Surg Pathol* 2005; **29**(7): 942-7.
24. Howat AJ, Beck S, Fox H, et al. Can histopathologists reliably diagnose molar pregnancy? *J Clin Pathol* 1993; **46**(7): 599-602.
25. Sebire NJ, Fisher RA, Rees HC. Histopathological diagnosis of partial and complete hydatidiform mole in the first trimester of pregnancy. *Pediatr Dev Pathol* 2003; **6**(1): 69-77.
26. Grinschgl I, Mannweiler S, Holzapfel-Bauer M, Pferschy U, Hoefler G, Guertl B. The role of morphology in combination with ploidy analysis in characterizing early gestational abortion. *Virchows Arch* 2013; **462**(2): 175-82.
27. Ronnett BM, DeScipio C, Murphy KM. Hydatidiform moles: ancillary techniques to refine diagnosis. *Int J Gynecol Pathol* 2011; **30**(2): 101-16.
28. Chen KH, Hsu SC, Chen HY, Ng KF, Chen TC. Utility of fluorescence in situ hybridization for ploidy and p57 immunostaining in discriminating hydatidiform moles. *Biochem Biophys Res Commun* 2014; **446**(2): 555-60.
29. Castrillon DH, Sun D, Weremowicz S, Fisher RA, Crum CP, Genest DR. Discrimination of complete hydatidiform mole from its mimics by immunohistochemistry of the paternally imprinted gene product p57KIP2. *Am J Surg Pathol* 2001; **25**(10): 1225-30.
30. Cheung AN. Pathology of gestational trophoblastic diseases. *Best Pract Res Clin Obstet Gynaecol* 2003; **17**(6): 849-68.
31. Bentley RC. Pathology of gestational trophoblastic disease. *Clin Obstet Gynecol* 2003; **46**(3): 513-22.
32. Wells M. The pathology of gestational trophoblastic disease: recent advances. *Pathology* 2007; **39**(1): 88-96.
33. Shih IM, Kurman RJ. The pathology of intermediate trophoblastic tumors and tumor-like lesions. *Int J Gynecol Pathol* 2001; **20**(1): 31-47.
34. Deavers MT, Kalhor N, Silva EG. Diagnostic problems with trophoblastic lesions. *Arch Pathol Lab Med* 2008; **132**(2): 168-74.
35. Fukunaga M. Early partial hydatidiform mole: prevalence, histopathology, DNA ploidy, and persistence rate. *Virchows Arch* 2000; **437**(2): 180-4.
36. Steigrad SJ. Epidemiology of gestational trophoblastic diseases. *Best Pract Res Clin Obstet Gynaecol* 2003; **17**(6): 837-47.

Chapter 3

Serum human chorionic gonadotropin normogram for the detection of gestational trophoblastic neoplasia

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ABSTRACT

Objective: To develop a serum hCG normogram for both uneventful complete and partial hydatidiform moles in the first-trimester ultrasound era.

Methods: An hCG normogram for both complete and partial hydatidiform moles was constructed, based on 639 patients with uneventful serum hCG regression after evacuation between 1990 and 2014. Serum hCG was measured by an in-house developed radioimmunoassay (RIA), detecting both intact hCG and free β-subunit. It has been in use for all serum measurements sent to the Dutch Central Registry for Hydatidiform Moles since 1977.

Results: Since introduction of routine first-trimester ultrasonography lower pre-evacuation and follow-up serum hCG concentrations were observed. When compared to complete hydatidiform moles, patients with a partial hydatidiform mole had significantly lower pre-evacuation serum hCG concentration (median 4400 ng/mL and 875 ng/mL, respectively, $P<0.001$) and earlier hCG normalization (median seven weeks and six weeks, respectively, $P<0.001$) but higher gestational age (mean 11.5 weeks and 13.0 weeks, respectively, $P<0.001$). For both complete and partial hydatidiform moles, 95% of patients reached normal serum hCG concentrations within fourteen weeks after evacuation.

Conclusions: A normogram for the detection of GTN was developed for complete and partial hydatidiform moles. Although interesting from a scientific perspective, the small divergence in hCG regression between complete and partial hydatidiform moles will be of little importance in clinical practice, as actual differences in regression will encompass just days. To promote clarity and unity in daily practice we therefore propose a combined normogram, to be used as a reference guideline for follow-up after evacuation of a hydatidiform mole. This normogram will be compliant with patients in today's clinical practice.

INTRODUCTION

The glycoprotein hormone human chorionic gonadotropin (hCG), produced by trophoblastic tissue, is a sensitive marker for monitoring trophoblastic activity in pregnancy and gestational trophoblastic disease (GTD). In post-molar gestational trophoblastic neoplasia (GTN), trophoblastic activity remains after evacuation of a hydatidiform mole, expressed by a plateau or rise in serum hCG concentration [1,2].

The International Federation of Obstetrics and Gynecology (FIGO) defines post-molar GTN as: 1) a plateau of hCG concentration in 4 consecutive blood samples over a period of 3 weeks, 2) a rise of hCG concentration for 3 consecutive samples over a period of 2 weeks, or 3) persistence of detectable hCG concentration 6 months after evacuation [3]. An elevated but falling hCG concentration 6 months after evacuation is no longer an absolute criterion, because with continuous surveillance, in all these patients spontaneous serum hCG normalization eventually occurred [4].

Follow-up after evacuation of a hydatidiform mole with serial serum hCG measurements is essential, as patients with GTN require further chemotherapeutic treatment [5-8]. Reference by a normogram allows close monitoring of all patients and facilitates early identification of those developing GTN [9]. Post-molar GTN occurs in approximately 15-20% of complete hydatidiform moles (CHM) following evacuation and occurs less frequently in partial hydatidiform moles (PHM), with approximately 0.5-1% [6].

In 1993 Yedema et al. [9] constructed a normogram, based on 130 patients with uneventful hCG regression following a complete hydatidiform mole between 1977 and 1989. At that time, hydatidiform moles typically presented in the second trimester with classic clinical signs of vaginal bleeding, uterine enlargement and hyperemesis [10-12]. Since the early 1990s however, ultrasonography is widely introduced as part of routine first-trimester clinical management. Consequently, hydatidiform mole patients are generally diagnosed at an earlier gestational age, before classical symptoms have developed. As a result, lower hCG levels at time of evacuation and earlier hCG normalization are expected today [11,13,14].

Furthermore, although the normogram as presented by Yedema et al. [9] has been used for both complete and partial hydatidiform moles, the normogram was solely based

on uneventful regression after complete hydatidiform moles and similar behavior of hCG regression in partial hydatidiform moles was only presumed. Complete and partial hydatidiform moles however comprise distinct features in terms of genetics, histology and clinical presentation [15-17], raising the question whether the presumption of similar hCG regression for both complete and partial moles is legitimate. Complete hydatidiform moles are characterized by uniform enlarged villi, diffuse hyperplasia and varying degrees of atypia, in absence of fetal tissue [15,18]. Genetically a complete hydatidiform mole is typically diploid, with all chromosomes from paternal origin [1,19]. In contrast, most partial hydatidiform moles have a triploid karyotype, fetal parts may be present, villi may vary in size and shape and hyperplasia is only focal with mild atypia [1,15,19,20].

Aim of the present study was to develop a serum hCG normogram for both uneventful complete and partial hydatidiform moles, applicable as a reference in the first-trimester ultrasound era. The possible benefit of separate normograms for both complete and partial hydatidiform moles in daily clinical practice was evaluated.

METHODS

Patients

The Dutch Central Registry for Hydatidiform moles at the Radboud University Medical Center Nijmegen (Radboudumc) was established in 1977. This voluntary registry serves as an epidemiological database and provides a nationwide centralized hCG measurement service for gynaecologists. Between 1977 and 2014, 4586 patients were registered. Since routine first-trimester ultrasonography was gradually introduced in the early 1990s, likely affecting mean gestational age and pre-evacuation serum hCG, for the present study patients were included from 1990 onwards. The following exclusion criteria were applied: diagnosis of GTN, hysterectomy or second curettage performed following hydatidiform mole, recurrence of disease after normalization, new pregnancy during follow-up and patients for whom hCG serum measurement performed at the central registry was either limited to less than 2 measurements or unavailable.

GTN was defined according to the FIGO 2000 guideline. In line with the Dutch guidelines, the following criterion was added to this definition: at least one of the values should exceed the 95th percentile of an hCG normogram of uneventful hCG decline as constructed by Yedema et al. [9].

All hCG measurements were performed using an in-house developed radioimmunoassay (RIA), based on polyclonal antibodies raised in rabbits [21]. This assay has been utilized centrally for all measurements in sera sent to the Dutch Central Registry for Hydatidiform Moles since 1977 and was described and likewise employed for construction of the normogram as presented by Yedema et al. in 1993 [9]. The assay is designed to specifically detect both intact hCG and free β-subunit. A highly purified hCG β-subunit preparation labeled with Iodine-125 was used as a tracer. The assay was calibrated with the third International Standard for hCG (WHO, 75-537). The cutoff value for normal hCG levels was established at 2 ng/mL, representing the cutoff serum concentration at 95% specificity as found in postmenopausal women [21].

Statistics

Serial hCG levels were pooled per 'week since evacuation' to obtain sufficient data points: i.e., week 0 represents the day of evacuation up to day 6 post-evacuation. Results were log-transformed to calculate the 5th, 50th and 95th percentile cutoffs per week post evacuation and subsequently plotted into a normogram. hCG normalization time was defined as the time in weeks from uterine evacuation of the mole until the time of the first normal hCG value. Differences in parametrical data were assessed by two-tailed student's *t* test. Non-parametric data were compared using two-tailed Mann-Whitney *U* test. Analyses were carried out using Microsoft Office Excel 2007 and SPSS for Windows (version 22).

RESULTS

Between 1990 and 2014, 324 patients diagnosed with complete hydatidiform mole and 315 patients with partial hydatidiform mole were eligible for further analyses. A total number of 5088 serum hCG measurements were used, with a median of eight measurements per patient. Pre-evacuation serum hCG measurements were available in 121 cases. Median pre-evacuation serum hCG was 3800 ng/mL (minimum 48 ng/

mL, maximum 35,000 ng/mL). Median half-life in the first part of hCG regression (week 0-3) was 3.0 days (minimum 2.3, maximum 6.4 days). In the second part of hCG regression (week 3-6) median half-life was 7.4 days (minimum 3.0, maximum 24.2 days). Figure 1 represents the serum hCG normogram for all uneventful hydatidiform moles. Some typical serum hCG courses of both patients with spontaneous regression and post-molar GTN are plotted in Figure 2. As shown, spontaneous regression after initial rise, even in combination with hCG levels exceeding the 95th percentile line can occasionally occur.

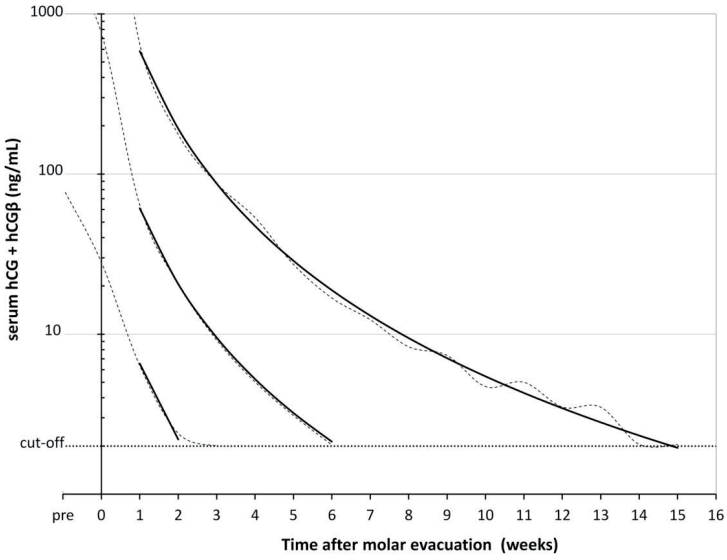

Figure 1. Fifth, 50th and 95th percentile lines of serum human chorionic gonadotropin (hCG) regression after evacuation of complete and partial hydatidiform moles. The three dashed lines correspond to the calculated 5th, 50th and 95th percentile, based on the available dataset of serum hCG in all patients with uneventful regression, the continuous lines indicate the smoothed trend lines. The serum hCG cutoff concentration of 2 ng/mL is presented by the horizontal dotted line.

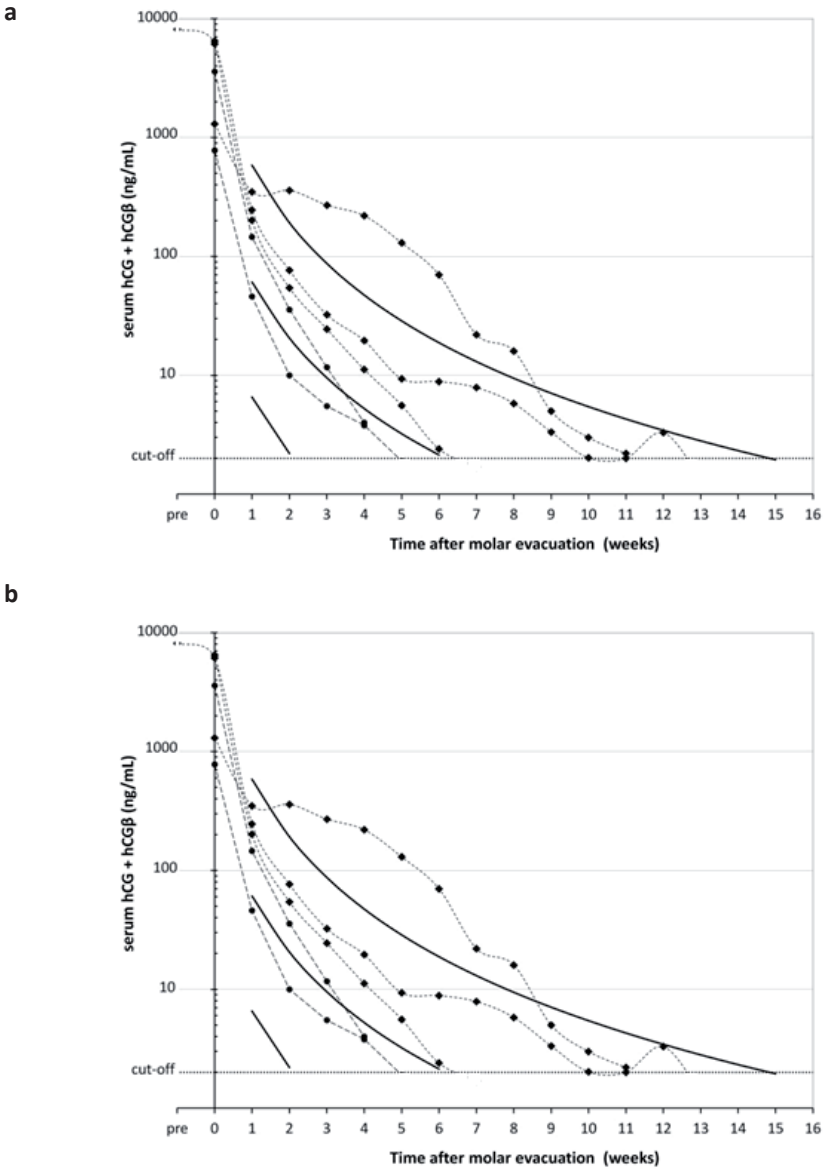

Figure 2. Some examples of serum hCG regression after evacuation of a hydatidiform mole. a) spontaneous regression after hydatidiform moles, b) serum hCG course in patients with gestational trophoblastic neoplasia before start of therapy. The horizontal dotted line represents the serum hCG cutoff concentration of 2 ng/mL

Clinical features for both complete and partial hydatidiform moles are listed in Table 1. Gestational age at time of evacuation was significantly lower in patients with a complete hydatidiform mole compared to patients with a partial hydatidiform mole (mean 11.5 weeks and 13.0 weeks respectively, P<0.001). Patients with a complete hydatidiform mole had significantly higher pre-evacuation serum hCG levels than patients with a partial hydatidiform mole (median 4400 ng/mL and 875 ng/mL, respectively, P<0.001). Furthermore, later normalization of serum hCG was seen in patients with complete hydatidiform moles when compared to partial hydatidiform moles (median

Table 1. Clinical features of patients with complete and partial hydatidiform moles

	Complete Hydatidiform Mole		Partial Hydaditidorm Mole		P
	N	*Mean (95% CI)*	*N*	*Mean (95% CI)*	
Maternal Age, years	323	29.8 (29.1-30.5)	315	30.2 (29.7-30.8)	0.349*
Gestational age, weeks	256	11.5 (11.1-11.9)	252	13.0 (12.6-13.5)	<0.001*
	N	*Median (min-max)*	*N*	*Median (min-max)*	
Pre-evacuation hCG (ng/mL)	89	4400 (133-35,000)	32	875 (48-8700)	<0.001+
hCG disappearance time (weeks)	284	7 (2-26)	286	6 (0-25)	<0.001+
hCG half-life first phase (days)[a]	69	3.0 (2.3-6.4)	16	3.1 (2.4-4.9)	0.822 +
hCG half-life second phase (days)[b]	145	6.9 (3.0-22.4)	81	7.9 (3.1-24.2)	<0.01 +

* Student's *t* test

\+ Mann-Whitney *U* test

[a] Week 0-3 since evacuation

[b] Week 3-6 since evacuation

seven weeks and six weeks respectively, P<0.001). hCG half-life in the second phase of regression however was significantly shorter in patients with complete hydatidiform moles when compared to patients with partial hydatidiform moles (median 6.9 and 7.9 days respectively, P<0.01). Median serum hCG levels per week after evacuation are shown in Table 2. For both complete and partial hydatidiform moles, 95% of the patients reached normal serum hCG levels within fourteen weeks since evacuation (Table 3), demonstrating overall small divergences.

Figure 3 represents a comparison between the serum hCG normogram of all uneventful complete hydatidiform moles in our study cohort and the historic cohort, as presented by Yedema et al. in 1993 [9]. When considering serum hCG levels per week since evacuation, compared to the historic cohort of Yedema et al. regression in our study group has shifted substantially to the left.

Table 2. Serum hCG levels per week after uterine evacuation for patients with complete and partial hydatidiform moles

Weeks[a]	**Complete Hydatidiform Mole**		**Partial Hydaditidorm Mole**		
	N	*Median hCG in ng/mL (min-max)*	*N*	*Median hCG in ng/mL (min-max)*	*P* [b]
Pre-evacuation	89	4400 (133-35,000)	32	875 (48-8700)	<0.001
0	82	1181 (5-82,000)	32	242 (6-77,000)	0.013
1	169	105 (5-960)	74	24 (1-1800)	<0.001
2	202	33 (1-1300)	127	12 (1-1400)	<0.001
3	206	14 (1-1200)	156	5 (1-527)	<0.001
4	201	7 (1-8450)	149	3 (1-150)	<0.001
5	208	4 (1-770)	154	2 (1-120)	<0.001

[a] Weeks since evacuation

[b] Mann-Whitney *U* test

Table 3. Cumulative percent rate of patients with hCG normalization after uterine evacuation

Cumulative percentage	**1%**	**2.5%**	**5%**	**50%**	**95%**	**97.5%**	**99%**
Complete Hydatidiform Mole							
Weeks after evacuation	2	2	3	7	14	19	23
Partial Hydatidiform Mole							
Weeks after evacuation	1	1	2	6	14	17	22

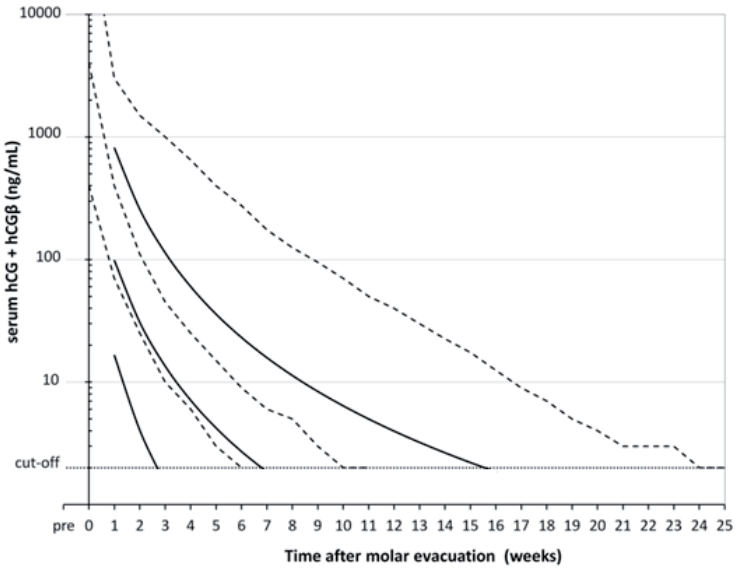

Figure 3. Fifth, 50th and 95th percentile lines of serum human chorionic gonadotropin (hCG) regression after evacuation of complete hydatidiform moles in cohort Yedema 1993 [(9)] (dashed lines) and present cohort (continuous lines) compared. The horizontal dotted line represents the serum hCG cutoff concentration of 2 ng/mL.

DISCUSSION

A serum hCG normogram for both uneventful complete and partial hydatidiform moles was constructed, applicable as a reference in the first-trimester ultrasound era. We observed lower hCG regression curves and earlier hCG normalization in the present study compared to the normogram formerly presented by our group in 1993 [9]. Earlier diagnosis aided by routine ultrasonography in the first trimester of pregnancy has likely resulted in a left-time shift of serum hCG regression in the present study, when compared to the normogram presented by Yedema et al. [9].

Thus far, regression was solely based on uneventful regression after complete hydatidiform moles and similar behavior of serum hCG regression for partial hydatidiform moles was merely presumed. The distinct genetic, histological and clinical characteristics of partial hydatidiform moles, however, requires an evaluation of a separate normogram and its clinical value in daily practice. Interestingly, patients with partial hydatidiform moles presented with lower pre-evacuation serum hCG levels and earlier hCG normalization time. This cannot merely be explained by an earlier diagnoses and evacuation of hydatidiform mole in these patients, as gestational age in this group was in fact higher when compared to patients with complete hydatidiform moles. The relative delay in evacuation in patients with partial hydatidiform moles may be explained by the typical features of partial hydatidiform moles on ultrasonography. Partial hydatidiform moles may present with fetal parts on ultrasonography in contrast with complete hydatidiform moles, where fetal parts are usually absent. Confirmation of a non vital, pathologic pregnancy may therefore be delayed [19].

In addition, the presence of different hCG subtypes may influence the hCG concentration measured and explain differences in hCG determination between partial and complete hydatidiform moles [22].

hCG is a heterogeneous molecule, produced by trophoblastic tissue during pregnancy [23]. Beside intact hCG, consisting of an α- and β-subunit , other forms of hCG are present in serum such as hyperglycosylated hCG, nicked hCG, free β-subunit, free α-subunit, nicked free β-subunit, and β-core fragments [22]. The production of subunits is under stringent physiological control in normal pregnancy and likely differs in pathological events such as a hydatidiform mole. Berkowitz et al. suggested an association be-

tween the percentage of free hCGβ and level of differentiation and hyperplasia of the trophoblast [24]. Considering the lower degree of hyperplasia in partial hydatidiform moles, one could hypothesize that in the case of partial moles, less free hCGβ is produced as compared to complete moles [24,25].

Although interesting from a scientific perspective, the small divergence in hCG regression between both complete and partial hydatidiform moles will be of minor importance in routine clinical practice, as actual differences in regression will encompass just days. Considering the normogram as a guideline during follow-up, a possible delay for patients with partial hydatidiform moles therefore yields no therapeutic consequences. Use of one normogram, as presented in Figure 1, will promote clarity and unity in daily practice, even before the type of molar pregnancy is confirmed by pathology. We note that the configuration of a normogram will be as dynamic as the patient group it affects, and the type of assay used. Changes in future practice may therefore require new adjustments over time.

CONCLUSION

In line with the internationally accepted criteria for diagnosis and treatment of GTN, the course of serum hCG (expressed by a plateau or rise) should be included in the decision to start chemotherapeutic treatment [26,27]. We constructed a serum hCG normogram for both uneventful complete and partial hydatidiform moles, updating the normogram as presented by Yedema et al. [9], which is applicable as a reference in the first-trimester ultrasound era. The normogram can assist follow-up of patients with both complete and partial hydatidiform moles after evacuation and support detection of post-molar GTN. Especially in patients with poor compliance to follow-up, this may be of vital importance.

REFERENCES

1. Lurain JR. Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. *Am J Obstet Gynecol* 2010; **203**(6): 531-9.
2. Seckl MJ, Sebire NJ, Fisher RA, et al. Gestational trophoblastic disease: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24 Suppl 6**: vi39-50.
3. Benedet JL, Bender H, Jones H, 3rd, Ngan HY, Pecorelli S. FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. FIGO Committee on Gynecologic Oncology. *Int J Gynaecol Obstet* 2000; **70**(2): 209-62.
4. Mangili G, Lorusso D, Brown J, et al. Trophoblastic disease review for diagnosis and management: a joint report from the International Society for the Study of Trophoblastic Disease, European Organisation for the Treatment of Trophoblastic Disease, and the Gynecologic Cancer InterGroup. *Int J Gynecol Cancer* 2014; **24**(9 Suppl 3): S109-16.
5. Berkowitz RS, Goldstein DP. Clinical practice. Molar pregnancy. *N Engl J Med* 2009; **360**(16): 1639-45.
6. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet* 2010; **376**(9742): 717-29.
7. Feltmate CM, Batorfi J, Fulop V, Goldstein DP, Doszpod J, Berkowitz RS. Human chorionic gonadotropin follow-up in patients with molar pregnancy: a time for reevaluation. *Obstet Gynecol* 2003; **101**(4): 732-6.
8. Hancock BW, Nazir K, Everard JE. Persistent gestational trophoblastic neoplasia after partial hydatidiform mole incidence and outcome. *J Reprod Med* 2006; **51**(10): 764-6.
9. Yedema KA, Verheijen RH, Kenemans P, et al. Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. *Am J Obstet Gynecol* 1993; **168**(3 Pt 1): 787-92.
10. Curry SL, Hammond CB, Tyrey L, Creasman WT, Parker RT. Hydatidiform mole: diagnosis, management, and long-term followup of 347 patients. *Obstet Gynecol* 1975; **45**(1): 1-8.
11. Soto-Wright V, Bernstein M, Goldstein DP, Berkowitz RS. The changing clinical presentation of complete molar pregnancy. *Obstet Gynecol* 1995; **86**(5): 775-9.
12. Hou JL, Wan XR, Xiang Y, Qi QW, Yang XY. Changes of clinical features in hydatidiform mole: analysis of 113 cases. *J Reprod Med* 2008; **53**(8): 629-33.
13. Jauniaux E, Nicolaides KH. Early ultrasound diagnosis and follow-up of molar pregnancies. *Ultrasound Obstet Gynecol* 1997; **9**(1): 17-21.
14. Kerkmeijer LG, Massuger LF, Ten Kate-Booij MJ, Sweep FC, Thomas CM. Earlier diagnosis and serum human chorionic gonadotropin regression in complete hydatidiform moles. *Obstet Gynecol* 2009; **113**(2 Pt 1): 326-31.
15. Bentley RC. Pathology of gestational trophoblastic disease. *Clin Obstet Gynecol* 2003; **46**(3): 513-22.
16. Ronnett BM, DeScipio C, Murphy KM. Hydatidiform moles: ancillary techniques to refine diagnosis. *Int J Gynecol Pathol* 2011; **30**(2): 101-16.
17. Nguyen NM, Slim R. Genetics and Epigenetics of Recurrent Hydatidiform Moles: Basic Science and Genetic Counselling. *Current obstetrics and gynecology reports* 2014; **3**: 55-64.
18. Hui P, Martel M, Parkash V. Gestational trophoblastic diseases: recent advances in histopathologic diagnosis and related genetic aspects. *Adv Anat Pathol* 2005; **12**(3): 116-25.

19. Wells M. The pathology of gestational trophoblastic disease: recent advances. *Pathology* 2007; **39**(1): 88-96.
20. Buza N, Hui P. Immunohistochemistry and other ancillary techniques in the diagnosis of gestational trophoblastic diseases. *Semin Diagn Pathol* 2014; **31**(3): 223-32.
21. Thomas CM, Segers MF, Houx PC. Comparison of the analytical characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. *Ann Clin Biochem* 1985; **22 (Pt 3)**: 236-46.
22. van Trommel NE, Sweep FC, Schijf CP, Massuger LF, Thomas CM. Diagnosis of hydatidiform mole and persistent trophoblastic disease: diagnostic accuracy of total human chorionic gonadotropin (hCG), free hCG {alpha}- and {beta}-subunits, and their ratios. *Eur J Endocrinol* 2005; **153**(4): 565-75.
23. Thomas CM, Reijnders FJ, Segers MF, Doesburg WH, Rolland R. Human choriogonadotropin (hCG): comparisons between determinations of intact hCG, free hCG beta-subunit, and "total" hCG + beta in serum during the first half of high-risk pregnancy. *Clin Chem* 1990; **36**(4): 651-5.
24. Berkowitz R, Ozturk M, Goldstein D, Bernstein M, Hill L, Wands JR. Human chorionic gonadotropin and free subunits' serum levels in patients with partial and complete hydatidiform moles. *Obstet Gynecol* 1989; **74**(2): 212-6.
25. Hay DL. Histological origins of discordant chorionic gonadotropin secretion in malignancy. *J Clin Endocrinol Metab* 1988; **66**(3): 557-64.
26. Ngan HY, Bender H, Benedet JL, et al. Gestational trophoblastic neoplasia, FIGO 2000 staging and classification. *Int J Gynaecol Obstet* 2003; **83 Suppl 1**: 175-7.
27. Committee FO. FIGO staging for gestational trophoblastic neoplasia 2000. FIGO Oncology Committee. *Int J Gynaecol Obstet* 2002; **77**(3): 285-7.

Chapter 4

Early prediction of post-molar gestational trophoblastic neoplasia with the use of a single serum human chorionic gonadotropin measurement

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ABSTRACT

Objective: Development of a clinically suitable prediction tool for patients with complete hydatidiform moles, to enable early prediction of post-molar GTN based on a single serum hCG measurement.

Methods: Serum hCG levels taken between 1 and 4 weeks after evacuation were retrospectively evaluated for patients with uneventful serum hCG regression (n=319) and patients with diagnosis of post-molar GTN (n=149), treated between 1990 and 2014. Logistic regression was used to generate nomograms for every week after evacuation, based on serum hCG measurements at that given week. Performance was determined by concordance index and calibration curves.

Results: All nomograms had good to excellent ability to distinguish between patients who will develop post-molar GTN and patients with uneventful hCG regression, reflected by a *c*-index value increasing from 0.83 in week 1, to 0.95 in week 4.

Discussion: With our nomograms, we developed a simple and reliable tool to estimate the individualized risk of developing post-molar GTN, based on a single serum hCG measurement taken after evacuation. These nomograms can be used by clinicians to either reassure patients at low-risk of post-molar GTN or to identify high-risk patients. Early start of chemotherapeutic treatment may be beneficial in patients at high-risk of post-molar GTN, especially in case of poor compliance to follow-up.

INTRODUCTION

Gestational trophoblastic disease (GTD) comprises a spectrum of pregnancy-related disorders including the premalignant complete and partial hydatidiform moles and the malignant conditions of choriocarcinoma and placental site trophoblastic tumor [1,2]. The term gestational trophoblastic neoplasia (GTN) has been applied collectively to the malignant counterparts [3]. The glycoprotein hCG, produced by trophoblastic tissue, is a sensitive marker for monitoring trophoblastic activity in gestational trophoblastic disease (GTD) [2,4]. To enable early detection of those requiring further treatment, all patients with GTD should be subjected to hCG surveillance [5].

In post-molar GTN, trophoblastic activity persists after evacuation of a hydatidiform mole, expressed by a plateaued or increased serum hCG concentration [6,7]. Post-molar GTN occurs in approximately 15-20% of complete hydatidiform moles (CHM) and in 0.5-1% of partial hydatidiform moles (PHM) and implies further treatment [1,5,8-10].

To date, it is still unclear why some hydatidiform moles develop into post-molar GTN, while others show spontaneous regression after evacuation. Although much effort has been made to evaluate the predictive value of clinical characteristics, such as advanced maternal age, history of molar pregnancy and large ovarian cysts, these factors have shown to be only weak predictors of post-molar GTN [2,11,12]. Most studies have therefore focused on the tumor marker hCG. Several investigators have mathematically evaluated whether hCG regression patterns of patients who go into spontaneous remission after molar evacuation differ from those who develop post-molar GTN [13-17]. Others have discussed the potential to predict post-molar GTN with hCG ratios obtained at different intervals [18,19]. Although these studies underline the relevance of serum hCG in post-molar GTN, none of these findings resulted in a sufficiently accurate prediction tool to be used in daily practice [14,15,17-19].

Few authors have discussed the possibility to predict post-molar GTN for patients with a complete hydatidiform mole, using a single serum hCG measurement after evacuation [18,20]. In some of these studies, pre-evacuation serum hCG levels and hCG levels taken in the first weeks after evacuation appeared predictive of post-molar GTN, but never with enough accuracy to guide individual management [12,18,20,21].

A tool that enables individualized and timely prediction of post-molar GTN could be used to either reassure patients at low-risk of post-molar GTN or to identify patients at high-risk of post-molar GTN.

Early initiation of chemotherapeutic treatment might be beneficial in patients at high-risk of post-molar GTN, especially when compliance to follow-up is poor. Aim of the present study was to develop a simple and reliable tool to enable early prediction of post-molar GTN based on a single serum hCG measurement.

METHODS

Patients

The Dutch Central Registry for Hydatidiform moles at the Radboud University Medical Center was established in 1977. This voluntary registry serves as an epidemiological database and provides a nationwide centralized hCG measurement service for gynecologists. Between 1977 and 2014, 4586 patients were registered. Routine first-trimester ultrasonography was gradually introduced in the early 1990s, affecting mean gestational age at diagnosis and pre-evacuation serum hCG. In the present study, we therefore included patients diagnosed between 1990 and 2014. All patients with available serum hCG data, obtained in the first four weeks after evacuation of a complete hydatidiform mole and analyzed at our institution were evaluated. The following exclusion criteria were applied: hysterectomy or second curettage performed before diagnosis of GTN was made, recurrence of disease after normalization or a new pregnancy during follow-up.

GTN was defined according to the FIGO 2000 guideline (i.e., mola hydatidosa with serum hCG plateauing for three consecutive weeks or rising over a period of two consecutive weeks). In line with the Dutch guidelines, the following criterion was added to this definition: at least one of the values should exceed the 95th percentile of an hCG normogram of uneventful hCG decline as constructed by Yedema et al. [17]. Patients were divided into two groups: those with uneventful serum hCG regression after evacuation of molar pregnancy (non-GTN group) and patients with proven diagnosis of GTN (GTN-group).

All hCG measurements were performed using an in-house developed radioimmunoassay (RIA), based on polyclonal antibodies raised in rabbits [22]. This assay has been utilized centrally for all measurements in sera sent to the Dutch Central Registry for Hydatidiform Moles since 1977. The assay has been designed to specifically detect both intact hCG and free β-subunit. A highly purified hCG β-subunit preparation labeled with Iodine-125 was used as a tracer. The assay is calibrated with the third International Standard (IS) for Chorionic Gonadotropin (WHO, 75-537). The cutoff value for normal hCG levels was established at 2 ng/mL, representing the cut-off serum concentration at 95% specificity as found in postmenopausal women [22].

Serial hCG levels were log-transformed and pooled per 'week since evacuation' to obtain sufficient datapoints: i.e., week 0 represents the day of evacuation up to day 6 post-evacuation. For the GTN-group, the hCG measurements taken after start of curative therapy (chemotherapy, hysterectomy or a curative second curettage) were excluded from analysis.

Statistics

Logistic regression analysis was carried out to identify the predictive value of a single hCG measurement for the development of post-molar GTN. The hCG measurements were added as a continuous variable. Nomograms were subsequently generated using R version 3.2.4 with the rms packages (http://R-project-org) [23]. Weighting was derived from this analysis. Performance was assessed by evaluating model fit, discrimination and calibration. The discriminative ability of the prognostic models, or the ability to distinguish women with low-risk of post-molar GTN from women with high-risk of post-molar GTN, was expressed by means of the concordance index (*c*-index). For a binary outcome, *c* is identical to the area under the receiver-operating characteristics (ROC) curve (AUC) [24]. *C*-indices of 0.6-0.7, 0.7-0.8, 0.8-0.9 and 0.9-1 represent fair, good, very good and excellent discriminative models, respectively [25]. Calibration was assessed graphically by means of the R package rms [25]. Calibration refers to the agreement between the predicted probability for post-molar GTN and the observed probability in the dataset. For example, if we predict a 20% risk of post-molar GTN for a particular patient, the observed frequency of post-molar GTN for similar patients should be approximately 2 out of 10 patients. In a calibration plot, the predicted GTN probabilities (x-axis) are plotted against the observed GTN probabilities (y-axis). Ide-

ally, if predicted and observed probabilities agree over the whole range, the plots show a perfect line of equality.

The internal validity of the models was tested using the bootstrapping method in which the selection and estimation process was repeated 500 times. Each of these repetitions resulted in the creation of a new dataset (bootstrap sample) by drawing cases with replacement from the original data. A correction for optimism in the *c*-index was derived from the bootstrap method to yield a bias-corrected *c*-index. In addition, a shrinkage factor was estimated to correct for statistical over optimism. A correction for optimism is necessary when a regression model becomes more complex and may begin to describe the random error or noise instead of the true underlying relationship [26].

Baseline features were compared using two-tailed Mann-Whitney *U* test for non-normally distributed data and two-samples Student's *t* test for normally distributed data. Analyses were carried out using the statistical packages of R (version 3.2.4), Microsoft Office Excel 2007 and SPSS for Windows (version 22).

RESULTS

Baseline characteristics

Between 1990 and 2014, 319 patients diagnosed with uneventful serum hCG regression following complete hydatidiform mole (non-GTN group) and 149 patients with post-molar GTN (GTN-group) were eligible for further analyses. A total of 1310 serum hCG measurements were performed between 1 and 4 weeks after evacuation, with a median of three measurements per patient. Baseline characteristics of both groups are presented in Table 1. Patients with uneventful serum hCG regression had comparable gestational age at time of evacuation as patients with post-molar GTN (mean 11.6 weeks and 11.3 weeks, respectively) and comparable maternal age (mean 29.3 years and 30.6 years, respectively). Furthermore, patients with uneventful regression had significantly lower pre-evacuation serum hCG levels than patients with post-molar GTN (median 5,000 ng/mL and 12,000 ng/mL, respectively, $p<0.001$).

Table 1. Baseline features of patients with uneventful serum hCG regression and patients with post-molar gestational trophoblastic neoplasia [a]

	Non-GTN group		GTN-group		P
	N	*Mean (SD)*	*N*	*Mean (SD)*	
Maternal Age (years)	319	29.3 (6.9)	149	30.6 (7.0)	0.07[b]
Gestational age (weeks)	241	11.6 (3.2)	109	11.3 (3.2)	0.50[b]
	N	*Median (min-max)*	*N*	*Median (min-max)*	
Pre-evacuation hCG (ng/mL)	92	5,000 (300-35,000)	49	12,000 (4-120,000)	<0.001[c]
hCG week 1 (ng/mL)	192	107 (3-4,600)	94	430 (26-10,000)	<0.001[c]
hCG week 2	235	33 (2-1,300)	109	300 (23-19,000)	<0.001[c]
hCG week 3	234	15 (2-1,200)	112	487 (14-28,000)	<0.001[c]
hCG week 4	231	7 (2-8,450)	103	400 (18-29,000)	<0.001[c]

[a] Data shown for all patients with baseline features available

[b] Student's *t* test

[c] Mann-Whitney *U* test

Nomogram development

Nomograms were generated for week 1-4 to predict the post-molar GTN risk, based on the hCG measurement taken in that week (Figure 1). The hCG measurement (ng/

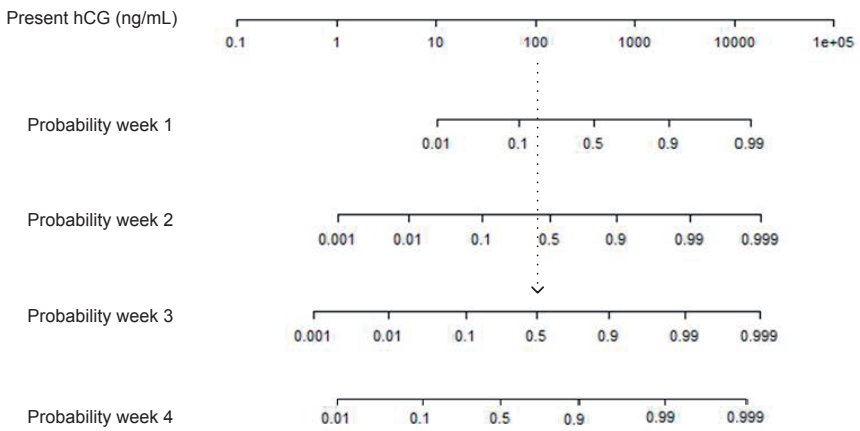

Figure 1. Nomograms for the prediction of post-molar GTN based on a single serum hCG measurement (ng/mL). The serum hCG measurement (ng/mL) is presented on a log-scale in the first row, which corresponds to the risk predictor of GTN in the row with that given weeknumber. The probability of post-molar GTN can be determined by drawing a vertical line connecting hCG at the top of the diagram and the risk scale at the bottom of the nomogram. For example, an hCG value of 100 ng/mL measured in week 3 corresponds to a 50% probability of post-molar GTN.

mL) is presented on a log-scale in the first row of Figure 1 and corresponds to the risk predictor of GTN in the row with that given week number. The probability of post-molar GTN can be determined by drawing a vertical line connecting present hCG at the top of the diagram and the risk scale at the bottom of the nomogram. For example, when a patient would present with a serum hCG measurement of 100 ng/mL in week 3, this corresponds with a 50% probability of post-molar GTN. In Table 2, an overview of hCG values and corresponding probabilities per week since evacuation is given.

Table 2. hCG values per week (ng/mL) with corresponding risk probabilities for post-molar GTN[a].

	hCG value (ng/mL)			
Probability	**Week 1**	**Week 2**	**Week 3**	**Week 4**
0.01	5	3	2	1
0.05	33	16	10	6
0.1	61	30	19	12
0.2	123	53	37	24
0.3	190	79	54	37
0.4	275	109	75	53
0.5	389	147	103	75
0.6	537	199	141	103
0.7	776	269	218	151
0.8	1202	398	286	234
0.9	2406	691	512	426
0.95	4365	1121	870	758
0.99	14000	3236	2570	2398

[a] The cutoff value for normal hCG levels is established at 2 ng/mL.

Discriminative accuracy and internal validation

In week 1 and 2, the *c*-index after correction of optimism is classified as very good (*c*-index 0.83; 95% CI 0.70-0.93 and *c*-index 0.90; CI 0.75-0.95, respectively). The *c*-index after correction of optimism for week 3 and 4 is classified as excellent (*c*-index 0.94; 95% CI 0.90-0.96 and *c*-index 0.95; 95% CI 0.92-0.97, respectively). This indicates that the models have good to excellent ability to discriminate between subjects who will or will not develop post-molar GTN.

The calibration plots, that provide a graphic representation of the agreement between prediction and actual outcome in our dataset are shown in Figure 2. For all

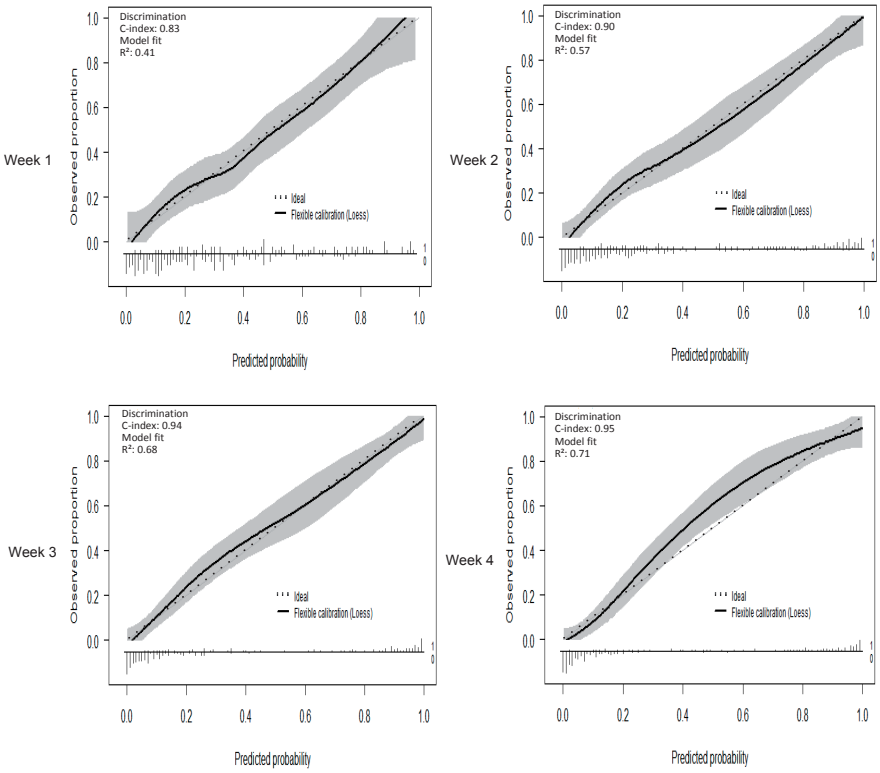

Figure 2. Calibration of all nomograms. Predicted GTN probabilities (x-axis) are plotted against the observed GTN probabilities (y-axis). Ideally, if predicted and observed probabilities agree over the whole range, the plots would show a perfect line of equality (dotted line). The black lines represent the apparent relation between the predicted and observed probabilities. The grey area represents the 95% confidence interval. *C*-index and R^2 are given for every model in the corresponding Figures.

models, there was no significant deviation in the calibration plots, indicating that the predicted risk of post-molar GTN was consistent with the actual post-molar GTN risk.

To evaluate whether risk prediction was steady over time, Figure 3 shows the consecutive probabilities for week 1 to 4 as derived from the nomograms, for patients either exceeding 80% post-molar GTN risk in the previous week or patients with a post-molar GTN risk remaining below 20% in the previous week. The Figures represent a selection of patients since serial weekly measurements were not always available. In week 1, 19 patients exceeded the 80% post-molar GTN risk, 18 of these patients

developed post-molar GTN in a later stage. A decline to ≤80% post-molar GTN risk in the following weeks was seen in 2 out of the 19 patients initially at high-risk of post-molar GTN. For 119 patients, the post-molar GTN risk was below 20% in week 1. Spontaneous regression was seen in 107 of these patients in a later stage. In nine out of the 119 patients at low-risk of post-molar GTN, a rise to ≥20% post-molar GTN risk was seen in a later stage. In week 2, 53 patients exceeded the 80% post-molar GTN risk, 49 of these patients developed post-molar GTN in a later stage. For one out

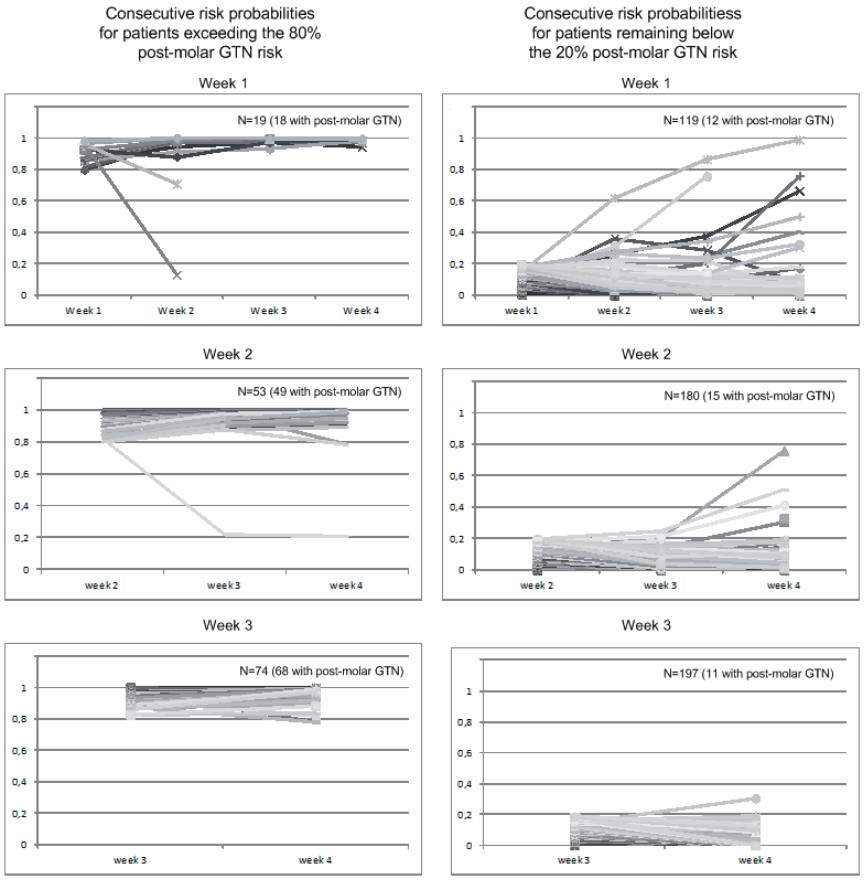

Figure 3. Evaluation of the consistency in risk prediction over time. The consecutive probabilities as derived from the nomograms are shown for patients exceeding the 80% post-molar GTN risk (left column) and patients with a post-molar GTN risk remaining below 20% (right column). The involved number of patients and post-molar GTN rate are shown in the corresponding Figures [a].

[a] The Figures represent a selection of patients since serial weekly measurements were not always available.

of the 53 patients at high-risk of post-molar GTN, a decline in the post-molar GTN risk to ≤80% was seen in the following weeks. The post-molar GTN risk was below 20% for 180 patients in week 2. Spontaneous regression was seen in 165 of these patients in a later stage. In four of these patients at low-risk of post-molar GTN, a rise to ≥20% post-molar GTN risk was seen. Finally, 74 patients had a post-molar GTN risk exceeding 80% in week 3, whereas 68 of these patients indeed developed post-molar GTN later on. Two patients at high-risk of post-molar GTN in week 3 showed a decline to a ≤80% post-molar GTN risk in week 4. A post-molar GTN risk below 20% in week 3 was seen in 197 patients. One hundred and eight-six of these patients showed a spontaneous regression in a later stage. Only one out of these 197 low-risk patients showed a rise to ≥20% post-molar GTN risk in week 4.

DISCUSSION

In the present study, we developed and internally validated nomograms to estimate the individual risk of post-molar GTN based on a single serum hCG measurement. Generally, the decline in serum hCG was considered a predominant factor in the prediction of post-molar GTN and several regression curves have been developed to facilitate early identification of post-molar GTN [15-17,27]. In these regression corridors, weekly serum hCG measurements are plotted in a regression curve, to establish whether a given hCG level is still within the normal range. These tools can be helpful as a reference guideline, but will not provide an individualized risk estimate.

Few studies have discussed the possibility to predict post-molar GTN based on serum hCG measurements taken in the first few weeks after evacuation [18,20,21]. Most of these studies primarily focused on pre-evacuation serum hCG and hCG taken in the first two weeks after evacuation. In studies presented by Kang et al. [18] and Mousavi et al. [21], the ratio between current hCG measurement and previous hCG measurement was recognized as a better predictive factor for post-molar GTN than serum hCG in week 1 or 2 alone. Based on ROC-curves, the optimal cut-off point was chosen and serum hCG measurements were divided into two categories to predict the risk of post-molar GTN. A limitation in this approach perhaps lies in the reduction of serum hCG measurements to two categories, whereas the association between serum hCG and post-molar GTN should probably be considered as a gradual scale. Additionally, only

measurements taken in the first two weeks after evacuation were evaluated in these analysis. Wolfberg et al. [20] presented a thorough evaluation of the predictive value of serum hCG measurements taken between 1 and 8 weeks after evacuation. Their analysis provides an important first step in the recognition of serum hCG measurements as a reliable predictor for post-molar GTN, providing clinicians with a rough estimate of the post-molar GTN risk. In their study, measurements were categorized into five categories to provide risk estimates per week. The risk estimates were therefore still coherent with, and limited to the chosen intervals.

With the use of nomograms as presented in this study, one can estimate the individualized risk of post-molar GTN as early as one week after evacuation, based on serum hCG alone. The nomograms are a practical and effective tool for prediction of post-molar GTN. With a *c*-index value ranging from 0.83 in week 1, to 0.95 in week 4, all nomograms had good to excellent ability to distinguish women with low-risk of post-molar GTN from women with high-risk of post-molar GTN and appeared steady over time.

In developing countries, early identification of patients with high-risk of post-molar GTN can be crucial, as resources are restricted and compliance of patients to follow-up may be limited [28]. Early identification of those at high-risk of post-molar GTN may however be equally valuable in developed countries. In this patient group, early start of curative therapy could ideally result in a shortened treatment period and earlier opportunity of a renewed pregnancy. Administration of chemotherapy to patients at high-risk of post-molar GTN, characterized by a risk exceeding 80% can therefore be considered under these circumstances when the benefit of empiric chemotherapy outweighs the risk of toxicity [29].

Considering the use of the nomograms presented in this study, a few limitations need to be addressed. First of all, despite the rather large sample size, serial weekly measurements were not always available, the models for week 1-4 were therefore based on a smaller subset of patients. Furthermore, hCG is a heterogeneous molecule comprising several subunits such as hyperglycosylated hCG, free β-subunit and free α-subunit [30]. Most hCG assays detect native hCG and its β-hCG subunits in equimolar amounts, whereas our in-house developed radioimmunoassay (RIA) preferably detects β-subunits over the native hCG. As a consequence, in the present form, the

models only apply to the specific assay at the Dutch Central Registry of Hydatidiform Mole. A validation of the concept presented in the present study is needed, before the tool can be used in a wider context.

CONCLUSION

We developed and internally validated nomograms to predict the risk of post-molar GTN using a single serum hCG measurement. With the use of these nomograms, clinicians can provide proper counseling of all complete hydatidiform mole patients and identify patients at high-risk of post-molar GTN. Early start of chemotherapeutic treatment can be considered in this patient group, especially when compliance to follow-up is poor.

REFERENCES

1. Seckl MJ, Sebire NJ, Fisher RA, et al. Gestational trophoblastic disease: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24 Suppl 6**: vi39-50.
2. Lurain JR. Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. *Am J Obstet Gynecol* 2010; **203**(6): 531-9.
3. Froeling FE, Seckl MJ. Gestational trophoblastic tumours: an update for 2014. *Curr Oncol Rep* 2014; **16**(11): 408.
4. Thomas CM, Reijnders FJ, Segers MF, Doesburg WH, Rolland R. Human choriogonadotropin (hCG): comparisons between determinations of intact hCG, free hCG beta-subunit, and "total" hCG + beta in serum during the first half of high-risk pregnancy. *Clin Chem* 1990; **36**(4): 651-5.
5. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet* 2010; **376**(9742): 717-29.
6. Committee FO. FIGO staging for gestational trophoblastic neoplasia 2000. FIGO Oncology Committee. *Int J Gynaecol Obstet* 2002; **77**(3): 285-7.
7. Ngan HY, Bender H, Benedet JL, et al. Gestational trophoblastic neoplasia, FIGO 2000 staging and classification. *Int J Gynaecol Obstet* 2003; **83 Suppl 1**: 175-7.
8. Berkowitz RS, Goldstein DP. Clinical practice. Molar pregnancy. *N Engl J Med* 2009; **360**(16): 1639-45.
9. Feltmate CM, Batorfi J, Fulop V, Goldstein DP, Doszpod J, Berkowitz RS. Human chorionic gonadotropin follow-up in patients with molar pregnancy: a time for reevaluation. *Obstet Gynecol* 2003; **101**(4): 732-6.
10. Hancock BW, Nazir K, Everard JE. Persistent gestational trophoblastic neoplasia after partial hydatidiform mole incidence and outcome. *J Reprod Med* 2006; **51**(10): 764-6.
11. Berkowitz RS, Bernstein MR, Laborde O, Goldstein DP. Subsequent pregnancy experience in patients with gestational trophoblastic disease. New England Trophoblastic Disease Center, 1965-1992. *J Reprod Med* 1994; **39**(3): 228-32.
12. Ayhan A, Tuncer ZS, Halilzade H, Kucukali T. Predictors of persistent disease in women with complete hydatidiform mole. *J Reprod Med* 1996; **41**(8): 591-4.
13. Schlaerth JB, Morrow CP, Kletzky OA, Nalick RH, D'Ablaing GA. Prognostic characteristics of serum human chorionic gonadotropin titer regression following molar pregnancy. *Obstet Gynecol* 1981; **58**(4): 478-82.
14. Shigematsu T, Kamura T, Saito T, Kaku T, Nakano H, Kinugawa N. Identification of persistent trophoblastic diseases based on a human chorionic gonadotropin regression curve by means of a stepwise piecewise linear regression analysis after the evacuation of uneventful moles. *Gynecol Oncol* 1998; **71**(3): 376-80.
15. Kim BW, Cho H, Kim H, et al. Human chorionic gonadotrophin regression rate as a predictive factor of postmolar gestational trophoblastic neoplasm in high-risk hydatidiform mole: a case-control study. *Eur J Obstet Gynecol Reprod Biol* 2012; **160**(1): 100-5.
16. Lybol C, Sweep FC, Ottevanger PB, Massuger LF, Thomas CM. Linear regression of postevacuation serum human chorionic gonadotropin concentrations predicts postmolar gestational trophoblastic neoplasia. *Int J Gynecol Cancer* 2013; **23**(6): 1150-6.

17. Yedema KA, Verheijen RH, Kenemans P, et al. Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. *Am J Obstet Gynecol* 1993; **168**(3 Pt 1): 787-92.
18. Kang WD, Choi HS, Kim SM. Prediction of persistent gestational trophobalstic neoplasia: the role of hCG level and ratio in 2 weeks after evacuation of complete mole. *Gynecol Oncol* 2012; **124**(2): 250-3.
19. van Trommel NE, Ngo Duc H, Massuger LF, et al. Early identification of persistent trophoblastic disease with serum hCG concentration ratios. *Int J Gynecol Cancer* 2008; **18**(2): 318-23.
20. Wolfberg AJ, Berkowitz RS, Goldstein DP, Feltmate C, Lieberman E. Postevacuation hCG levels and risk of gestational trophoblastic neoplasia in women with complete molar pregnancy. *Obstet Gynecol* 2005; **106**(3): 548-52.
21. Mousavi AS, Karimi S, Modarres Gilani M, Akhavan S, Rezayof E. Does Postevacuation beta -Human Chorionic Gonadotropin Level Predict the Persistent Gestational Trophoblastic Neoplasia? *ISRN Obstet Gynecol* 2014; **2014**: 494695.
22. Thomas CM, Segers MF, Houx PC. Comparison of the analytical characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. *Ann Clin Biochem* 1985; **22 (Pt 3)**: 236-46.
23. Harrell F. Regression modeling strategies: documentation for package 'rms' version 5.0-0. 2016.
24. Steyerberg EW, Vickers AJ, Cook NR, et al. Assessing the performance of prediction models: a framework for traditional and novel measures. *Epidemiology* 2010; **21**(1): 128-38.
25. Choi BC. Slopes of a receiver operating characteristic curve and likelihood ratios for a diagnostic test. *Am J Epidemiol* 1998; **148**(11): 1127-32.
26. Steyerberg EW, Eijkemans MJ, Harrell FE, Jr., Habbema JD. Prognostic modeling with logistic regression analysis: in search of a sensible strategy in small data sets. *Med Decis Making* 2001; **21**(1): 45-56.
27. Kerkmeijer LG, Massuger LF, Ten Kate-Booij MJ, Sweep FC, Thomas CM. Earlier diagnosis and serum human chorionic gonadotropin regression in complete hydatidiform moles. *Obstet Gynecol* 2009; **113**(2 Pt 1): 326-31.
28. Cagayan MS, Gacoba MC. Chemotherapy regimens used in the treatment of gestational trophoblastic neoplasia at Philippine General Hospital: treatment outcomes and toxicity. *J Reprod Med* 2006; **51**(11): 907-18.
29. Park TK, Kim SN, Lee SK. Analysis of risk factors for postmolar trophoblastic disease: categorization of risk factors and effect of prophylactic chemotherapy. *Yonsei Med J* 1996; **37**(6): 412-9.
30. van Trommel NE, Sweep FC, Schijf CP, Massuger LF, Thomas CM. Diagnosis of hydatidiform mole and persistent trophoblastic disease: diagnostic accuracy of total human chorionic gonadotropin (hCG), free hCG {alpha}- and {beta}-subunits, and their ratios. *Eur J Endocrinol* 2005; **153**(4): 565-75.

Chapter 5

The added value of hysterectomy in the management of gestational trophoblastic neoplasia

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ABSTRACT

Background: Despite the undoubted effectiveness of chemotherapeutic treatment in gestational trophoblastic neoplasia (GTN), problems related to toxicity of chemotherapy and chemo-resistant disease have led to reconsideration of the use of hysterectomy. Aim of the present study was to evaluate indications for and outcome of hysterectomy in patients with GTN in a nation-wide cohort.

Methods: Between 1977 and 2012, we identified all patients diagnosed with GTN and treated with hysterectomy from the Dutch national databases. Demographics, clinical characteristics and follow-up were recorded retrospectively.

Results: One hundred and nine patients (16.5% of all registered patients with GTN) underwent hysterectomy as part of their management for GTN. The majority of patients was classified as low-risk disease (74.3%), post-molar GTN (73.5%) and disease confined to the uterus (65.1%). After hysterectomy, complete remission was achieved in 66.2% of patients with localized disease and in 15.8% of patients with metastatic disease. For patients with localized disease, treated with primary hysterectomy, treatment duration was significantly shorter (mean 3.2 weeks and 8.0 weeks respectively, $p=0.01$) with lower number of administered chemotherapy cycles (mean 1.5 and 5.8 respectively, $p<0.01$) than patients in a matched control group.

Conclusion: In selected cases, a hysterectomy may be an effective means to either reduce or eliminate tumor bulk. Primary hysterectomy should mainly be considered in older patients with localized disease and no desire to preserve fertility, whereas patients with chemotherapy-resistant disease may benefit from additional hysterectomy, especially when disease is localized. For patients with widespread metastatic disease, the benefit of hysterectomy lies in the removal of chemotherapy-resistant tumor bulk with subsequent effect on survival.

INTRODUCTION

Gestational trophoblastic disease (GTD) comprises a spectrum of conditions ranging from the pre-malignant complete (CHM) and partial (PHM) hydatidiform moles to the malignant choriocarcinoma and placental site trophoblastic tumor (PSTT) [1,2]. The term gestational trophoblastic neoplasia (GTN) has been applied collectively to the malignant counterparts [3]. Post-molar GTN occurs in approximately 15-20% of complete hydatidiform moles and 0.5-1% of partial hydatidiform moles, characterized by plateauing or rising blood human chorionic gonadotrophin concentration (hCG).

The management of GTN can be considered a success story of modern medicine. Historically, hysterectomy was the treatment of choice in patients with GTN. In the early 1960s, outcome was poor with 5-year survival rate after hysterectomy of 41% in non-metastatic disease and 19% in metastatic disease [4]. The introduction of chemotherapy, recognition of risk factors allowing for individualized treatment and use of sensitive hCG assays as a valuable marker for monitoring disease are the main reasons for current treatment success [5]. Today, trophoblastic neoplasias are among the most curable malignancies, with cure rates approaching 90%, even in the presence of widespread disease [1,6,7].

Despite the excellent success of chemotherapy in most patients, problems related to chemo-resistant disease and toxicity have led to reconsideration of the use of hysterectomy in individualized cases. Hysterectomy with or without lymph node dissection remains the treatment of choice for PSTT, as these tumors appear fairly chemo-resistant with a propensity for lymphatic spread [8-11]. Secondary hysterectomy and metastasectomy (ie pulmonary resection, craniotomy, liver lobe resection) play a significant role in the management of chemo-resistant disease [1,12-14]. Response to first-line chemotherapy is estimated to be incomplete in 25% of low-risk patients and 20% of high-risk patients respectively [15]. Furthermore, surgical procedures may be inevitable in case of life-threatening hemorrhage [16,17]. A primary hysterectomy may be considered for perimenopausal patients without the desire to preserve fertility, ideally resulting in a reduction of administered cycles of chemotherapy and a subsequent reduction of toxic effects [13,17]. In addition, although the current study is mainly focused on GTN, it should be noted that hysterectomy may sometimes be considered as primary treatment for GTD [18].

Although several authors have assessed the role of hysterectomy in GTN, it generally involved small-size studies [4,12,13,17]. Apprehension of the possible benefits of hysterectomy is needed to facilitate the individual treatment decision in daily practice. In the present study, we evaluated indications for and outcome of hysterectomy in patients with GTN in a large nation-wide cohort. Considering the wide timeframe of our cohort, possible differences in indication for and outcome after hysterectomy were evaluated over time.

MATERIALS AND METHODS

Patients

Since 1977 patients with GTD are registered at the Dutch Central Registry for hydatidiform moles at the Radboud University Medical Center, Nijmegen. This voluntary registry serves mainly as an epidemiological database and provides an hCG assay service for gynecologists nationwide. Patients with GTN are treated in referral hospitals and are discussed in the Dutch Working Party on Trophoblastic Disease. Data on patients with hysterectomy performed between 1977 and 2012 were collected from the database and records of the Dutch Working Party meetings. Moreover, hospital records of all patients were reviewed for additional information. For each patient, we reviewed demographic characteristics, FIGO 2000 prognostic scoring criteria, indication for surgery, treatment regimen, response to treatment, histological diagnosis and follow-up of at least two-years. Nine patients with PSTT were excluded from further analysis, since management and prognosis for these patients differs from other GTN patients [8-10]. One hundred and nine patients were eligible for further analysis (Figure 1). A comparison between patients with hysterectomy performed before and after 1995 was made, to evaluate how indications for hysterectomy and outcome after hysterectomy may have changed over time. The cutoff point was chosen arbitrary, resulting in two equal timeframes.

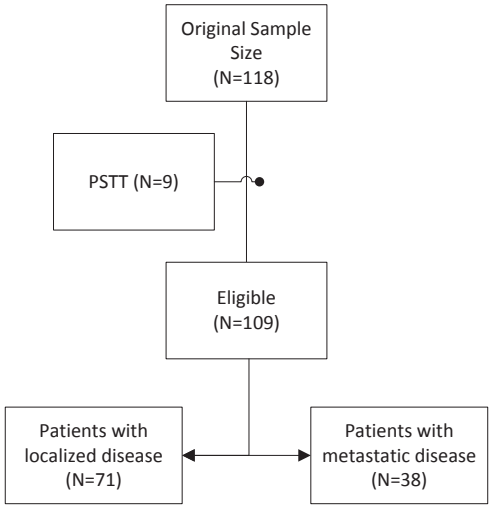

Figure 1. Flow diagram of patient sample

Definitions

GTN was diagnosed according to the FIGO 2000 criteria (ie, mola hydatidosa with serum hCG plateauing for three consecutive weeks or increasing over a period of two consecutive weeks). In line with the Dutch guidelines, the following criterion was added to this definition: at least one of the values should exceed the 95th percentile of an hCG normogram of uneventful hCG decline as constructed by Yedema et al. [19]. Chemotherapy-resistant disease was defined as a plateauing or rising serum hCG concentration in three consecutive weekly measurements. Resistance to therapy was assessed by serum hCG measurements, using an in-house developed radioimmunoassay (RIA) that detects both intact hCG and free β-subunit [20]. hCG follow-up was undertaken weekly until serum hCG was normal and then monthly until one year after completion of single-agent chemotherapy or two years after completion of multi-agent chemotherapy.

Protocols for the management of GTN

To select the appropriate treatment, all patients were classified into low- or high-risk disease using the Dutch risk classification. Risk classification with this system shows an extensive overlap with the more widely used FIGO 2000 [21]. Low-risk patients received single-agent methotrexate (MTX) with folinic acid rescue (50 mg intramuscular MTX

on days 1, 3, 5, and 7 and folinic acid 15 mg orally on days 2, 4, 6, and 8). Between 1977 and 1990 high-risk patients and patients with chemo-resistant disease were treated with multi-agent chemotherapy in various effective regimens. Since 1990, the EMA/CO chemotherapeutic regimen, consisting of etoposide, methotrexate, actinomycin D alternating weekly with cyclophosphamide and vincristine is recommended as treatment of choice in patients developing resistance, unmanageable toxicity or high-risk disease [22,23].

Statistical analysis

To evaluate the therapeutic effect of hysterectomy, a comparison between patients with hysterectomy and patients without hysterectomy was performed. Due to the retrospective nature of this cohort study, patients were not randomized prior to hysterectomy. To reduce the bias of possible confounding factors, we used the propensity score matching method as described by Austin [24]. In this analysis, patients treated with hysterectomy were matched one-to-one with patients without hysterectomy treatment, based on maternal age, antecedent pregnancy, interval between evacuation and treatment, and pre-treatment serum hCG. The performance of the propensity score model was evaluated by assessing whether any important relationship between both groups and the covariates remained after adjustment. With the excellent prognosis of GTN today, treatment duration and number of chemotherapy cycles needed to reach complete remission instead of survival were the outcome variables of choice.

Differences in parametrical data were assessed by two-tailed students *t* test. Non-parametric data were analyzed with two-tailed Mann-Whitney *U* test and Fisher-Freeman-Halton Test. Analyses were carried out using Microsoft Office Excel 2007 and SPSS for Windows (version 22).

RESULTS

From 1977 to 2012, a total of 713 patients diagnosed with GTN were registered at the Dutch Central Registry for Hydatidiform Moles or discussed at the Dutch Working derwent hysterectomy as part of their management for GTN. For 28 of these patients (25.7%), hysterectomy was performed with uni- or bilateral salpingo-oophorectomy.

Median age at start of treatment was 36 years (minimum 20, maximum 60), with the majority of cases classified as low-risk disease (74.3%), post-molar GTN (73.5%) and disease confined to the uterus (65.1%). Demographic and clinical characteristics for patients with localized disease and patients with metastatic disease are shown in Table 1. Metastatic disease predominantly involved lung or vaginal involvement. In 8

Table 1. Demographic and clinical characteristics of all patients with hysterectomy performed

	Localized disease N=71	**Metastatic disease N=38**		**Total Cohort N=109**
	Median (min-max)	Median (min-max)	P	Median (min-max)
Age (Years)a	37.0 (24-60)	34.0 (20-52)		36.0 (20-60)
Interval months from index pregnancy	1.0 (0-46)	1.0 (0-24)		1.0 (0-46)
Pretreatment 10log Serum hCG (ng/mL)	3.7 (1.04-5.73)	4.6 (0.3-5.95)		3.9 (0.3-5.95)
	Number (%)	Number (%)	P	Number (%)
Antecedent pregnancy			<0.01	
Term	6 (8.5%)	8 (21.1%)		14 (12.8%)
Hydatidiform Mole	62 (87.3%)	18 (47.4%)		80 (73.5%)
Abortion/unknown	3 (4.2%)	12 (31.5%)		15 (13.7%)
Site of metastasis			<0.01	
None	71 (100.0%)	-		71 (65.1%)
Vagina or Lung	-	25 (65.8%)		25 (22.9%)
Other metastasis b	-	13 (34.2%)		13 (11.9%)
Risk classification			<0.01	
Low-risk	64 (90.1%)	17 (44.7%)		81 (74.3%)
High-risk	7 (9.9%)	20 (52.6%)		27 (24.8%)
Unkown	-	1 (2.6%)		1 (0.9%)
Metastasectomy			<0.01	
Yes	-	8 (21.1%)		8 (7.3%)
No	71 (100.0%)	30 (78.9%)		101 (92.7%)
Pathology findings			<0.01	
Negative for trophoblastic disease	6/63 (9.5%)	8/35 (22.9%)		14/98 (14.3%)
	Number (%)	Number (%)	P	Number (%)
Follow-up a				
Complete remission following hysterectomy c	48/71 (67.6%)	6/38 (15.8%)	<0.05	54/109 (49.5%)
Complete remission after finishing treatment	66/68 (97.1%)	28/35 (73.7%)	<0.01	94/103 (91.3%)
Complete remission after 6 months	43/45 (95.6%)	20/27 (74.1%)	<0.05	63/72 (87.5%)
Complete remission after 12 months	35/38 (92.1%)	18/25 (47.4%)	<0.05	53/63 (84.1%)
Complete remission after 24 months	27/30 (90.0%)	16/23 (42.1%)	0.08	43/53 (81.1%)
Mortality	2/67 (3.0%)	7/34 (20.6%)		9/101 (8.9%)

[a] Follow-up is shown for all patients where follow-up information was available

[b] Other metastasis may be localized in kidney, gastro-intestinal tract, liver and or brain

[c] Compete remission after hysterectomy, achieved without further chemotherapy or surgery

cases (7.3%) a metastasectomy was additionally performed, mainly involving resection of pulmonary metastases. Nine patients (8.9%) died as a result of their GTN. In six of these cases, brain metastases causing intracranial hemorrhage was the eventual cause of death, other causes included sepsis following severe myelosuppression (n=1) and hypovolemic shock due to severe intra-abdominal hemorrhage (n=2). Twelve months after completion of treatment, 84.1% of patients with follow-up information available still showed no signs of recurrent disease.

Histological findings of the resected uterine specimen were negative for gestational trophoblastic disease in 14.3% of cases (Table 1). Severe complications as a result of the surgical procedure occurred in 4 cases (3.5%), consisting of cardiac symptoms requiring re-admittance (n=1), severe hemorrhage requiring surgical re-intervention (n=2) and post-operative infection requiring re-intervention (n=1).

In Table 2, a comparison of patient characteristics and outcome between patients with hysterectomy performed before and after 1995 is shown. When compared to patients in the first cohort, patients in the second cohort presented with a significantly older age (median 34.8 and 41.2 respectively, p<0.01) but comparable pre-treatment serum hCG (median 3.8 ng/mL and 4.0 ng/mL respectively, p=0.68) and interval (median both 1.0 months, p=0.20). The indications for hysterectomy and the complete remis-

Table 2. Characteristics, treatment indication and outcome between first and second cohort compared

	First Cohort [a] (N=58)	**Second cohort [b] (N=51)**	
	Median (min-max)	**Median (min-max)**	***P***
Age (years)	34.8 (20-60)	41.2 (25-55)	<0.01
Interval months from index pregnancy	1.0 (0-46)	1.0 (0-21)	0.20
Pretreatment ^{10}log Serum hCG (ng/mL)	3.8 (0.3-6.0)	4.0 (1.0-5.7)	0.68
	Number (%)	**Number (%)**	
High-risk disease	40/81 (49.4%)	17/27 (63.0%)	0.19
Indication hysterectomy			0.31
Primary treatment	13 (22.4%)	17 (33.3%)	
Chemo-resistant disease	39 (67.2%)	27 (52.9%)	
Hemorrhage	6 (10.3%)	7 (13.7%)	
Complete Remission after hysterectomy	25/51 (49.0%)	29/48 (60.4%)	0.16

[a] First cohort represents all patients treated with hysterectomy before 1995

[b] Second cohort refers to all patients treated with hysterectomy from 1995 onwards

sion rate following hysterectomy were not different between the first and second cohort (p=0.31 and p=0.16 respectively).

The three main reasons for hysterectomy were primary definitive treatment, chemo-resistant disease and life-threatening hemorrhage, which mainly involved intra-abdominal hemorrhage. Overall, 48 of the 71 patients with localized disease (67.6%) achieved complete remission after hysterectomy. The indication-specific response to hysterectomy for patients with localized disease is shown in Figure 2a. Complete remission after primary hysterectomy was seen in 47.8% of patients with

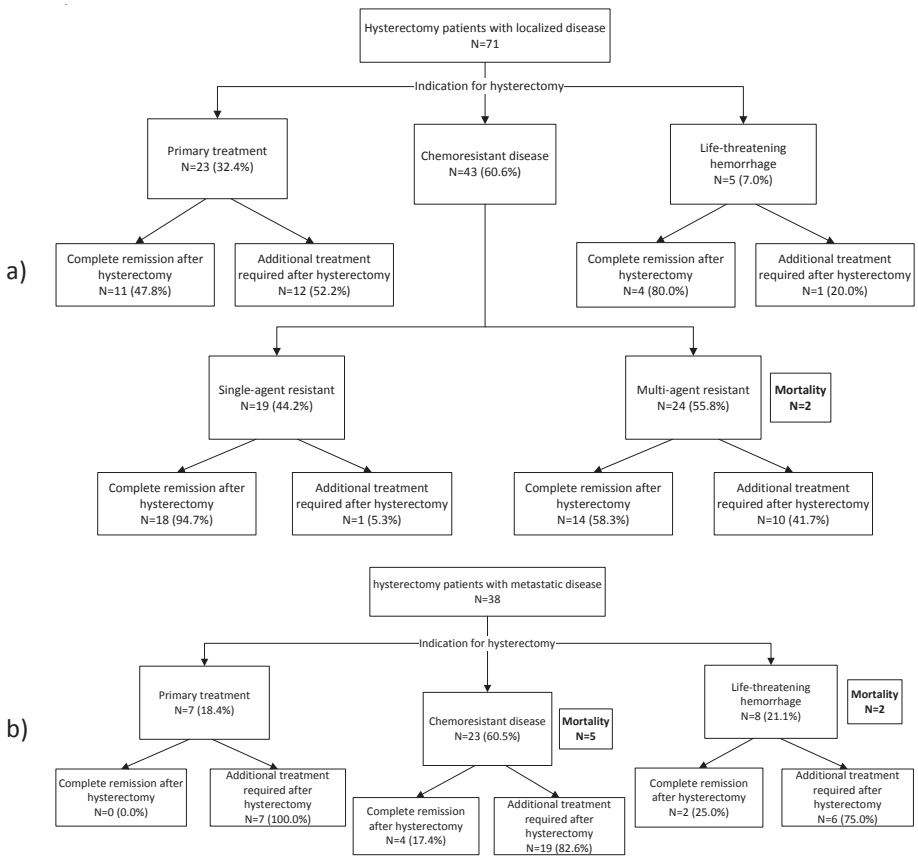

Figure 2. Indication and effect of hysterectomy for patients with GTN, a) patients with localized disease, b) patients with metastatic disease. The classification between localized and metastatic disease was made at start of treatment. Some of these patients may have presented with occult metastatic disease later in their treatment course.

localized disease. Complete remission after hysterectomy occurred more frequently in single-agent resistant patients than in multi-agent-resistant patients (94.7% and 58.3%, p<0.05).

When patients presented with metastatic disease, no patients achieved complete remission with primary hysterectomy alone, whereas only 6 out of 31 (19.4%) patients achieved complete remission after hysterectomy was done for chemo-resistant disease or life-threatening hemorrhage. The indication-specific response to hysterectomy for patients with metastatic disease is shown in Figure 2b.

In Table 3 treatment duration and number of chemotherapy cycles is compared between patients with localized disease, treated with primary hysterectomy and a one-to-one matched control group primarily treated with chemotherapy. Four subjects who did not have a reasonable match were excluded from further analysis. When compared to the matched control group, patients treated with primary hysterectomy for localized disease had a significantly shorter treatment duration (mean 8.0 weeks and 3.2 weeks respectively, p=0.01) and lower number of administered chemotherapy cycles (mean 5.8 and 1.5 respectively, p<0.01). To evaluate the performance of the propensity scoring model, a comparison of the covariates involved in the matching procedure was made between the study group and matched control group. The covariates did not significantly differ between both groups.

Table 3. Comparison of treatment duration and number of chemotherapy cycles between patients treated with primary hysterectomy for localized disease and a matched control group

	Primary hysterectomy performed N=19	**Matched Control group N=19**	
	Number	**Number**	***P***
Single-Agent administered	8/19	19/19	<0.01
Multi-Agent administered	1/19	1/19	1.00
	Mean (sd)	**Mean (sd)**	***P***
Treatment duration (weeks)	3.2 (3.2)	8.0 (4.8)	0.01
Number of courses			
Total	1.5 (2.6)	5.8 (2.9)	<0.01
Single-Agent	1.2 (2.1)	5.4 (2.1)	<0.01
Multi-Agent	0.2 (0.9)	0.4 (1.8)	0.69

For patients with a hysterectomy performed to control chemo-resistant disease or intra-abdominal hemorrhage, identification of a matched control group through propensity matching was not feasible, as their clinical characteristics widely differed.

DISCUSSION

In the present study we explored the added value of hysterectomy in patients with gestational trophoblastic neoplasia. Approximately one out of six GTN patients (16.5%) required hysterectomy during the course of their treatment to either remove tumor bulk or treat complications. After hysterectomy, complete remission was achieved in 67.6% of patients with localized disease and in 15.8% of patients with metastatic disease. Primary hysterectomy resulted in complete remission in 47.8% of cases when disease was localized. Comparison with a matched control group showed that a shorter treatment duration and lower number of chemotherapy cycles was needed to reach complete remission after primary hysterectomy.

Considering the wide timeframe of our cohort, possible differences in clinical characteristics, treatment indications and outcome were compared between the first and second cohort. In line with other studies, the groups had generally comparable characteristics with a similar distribution of treatment indications [25].

Other centers have published their experience on the management of GTN using hysterectomy with incidence ranging from 17% to 32% [25-30]. Higher incidence rates likely result from differences in available resources for treatment and follow-up, as some studies were conducted in developing countries [27]. In line with these other studies, main indications for hysterectomy in our patients with GTN were primary definitive treatment, chemo-resistant disease and life-threatening hemorrhage [12,25,27,28].

Since patients with metastatic disease present with distinct clinical features and a slightly less favorable prognosis than patients with localized disease, both groups were analyzed separately. Complete remission following hysterectomy was incidentally seen in patients with metastatic disease, in general the procedure was however less effective in patients with metastatic disease than in patients with localized disease.

Therefore, information on extra-uterine disease involvement seems important while considering treatment with hysterectomy.

Primary hysterectomy has been suggested in perimenopausal patients when fertility preservation is not required. Ideally this would result in a reduction of chemotherapy cycles needed to achieve complete remission. Reported results on the actual benefits are however conflicting [13,17,31,32]. In general the procedure is expected to be most beneficial in patients with localized disease, but primary hysterectomy may also be considered in selected patients with high-risk metastastic GTN and limited extra-uterine burden [33,34]. Hammond et al. reported that elective initial hysterectomy significantly reduced the duration of hospitalization and the number of chemotherapy cycles needed to achieve complete remission in both patients with and without metastases [31]. In the present study 47.8% of patients with localized disease had complete sustained remission following primary hysterectomy alone. When compared to a matched control group, treatment with primary hysterectomy resulted in a shorter treatment duration and lower number of chemotherapy cycles needed to reach complete remission, when disease was localized. Considering the performance of salpingo-oophorectomy in some patients, it should be noted that this procedure may have simultaneously removed occult metastatic disease. In patients with metastatic disease, primary hysterectomy was less effective and additional chemotherapeutic treatment was always indicated. Primary hysterectomy should therefore mainly be considered in patients over forty years of age, without extra-uterine burden or desire to preserve fertility.

In selected patients with persistent or recurrent high-risk GTN, hysterectomy is used to remove foci of chemotherapy-resistant disease [33]. In some cases, hysterectomy can reduce or eliminate the need for further chemotherapeutic treatment [31,35-37]. When disease was confined to the uterus, 94.7% of single-agent resistant cases was fully salvaged by hysterectomy, whereby the more aggressive multi-agent regimen could be avoided. Examining the role of hysterectomy in patients with metastatic disease, revealed a less favorable effect of hysterectomy, with 17.4% being fully salvaged with hysterectomy.

Usually, the presence of active disease outside the uterus will require additional management, though incidentally due to previous chemotherapeutic treatment these foci

may have resolved. As mentioned before, patients with widespread metastatic disease represent a selected group with recurrent or persistent chemo-resistant disease. Fatal outcome as a consequence of the disease, is predominantly seen in this particular group. Therefore, the primary benefit of hysterectomy in patients with metastatic disease is not a decline in administered cycles of multi-agent chemotherapy, but a reduction of chemo-resistant tumor bulk with subsequent effect on survival.

Finally, hysterectomy may play a role in therapy for high-risk disease as a means of dealing with life-threatening complications, such as intra-abdominal or vaginal hemorrhage [33]. Thirteen patients (11.9%) in our total study cohort required hysterectomy to control hemorrhage, mainly intra-abdominal. These patients can present with excessive bleeding and may therefore be haemodynamically unstable.

In the present study, no histological evidence of active trophoblastic disease in the uterus was found in 14.3% of cases. This was predominantly apparent in patients with chemo-resistant disease, when cure appeared to be challenging. In some of these cases, tumor foci might have been present on imaging as trophoblastic tumors have slow radiologic resolution, particularly in bigger masses [38]. Although it is imperative to perform imaging and assess the presence of uterine disease, one should therefore keep in mind that the volume of a trophoblastic tumor may not represent the proportion of viable cells present [2,39]. Positron emission tomography-computer tomography (PET CT) scanning could be a helpful tool to discriminate active versus necrotic tumors [40].

Women treated with hysterectomy represent a specific subset of patients and may not represent the average patient with GTN. Depending on the indication for surgery, these patients may present with larger tumor bulk when treated for chemo-resistant disease or poorer physical fitness when presented with disease related complications such as hemorrhage. To enable the evaluation of possible therapeutic effects of hysterectomy, we strived to perform a comparison between patients treated with hysterectomy and a matched control group. Unfortunately, due to the wide differences in clinical characteristics, no consistent control group could be matched to the hysterectomy patients with chemo-resistant disease or hemorrhage and patients with metastatic disease who were primarily treated with hysterectomy.

CONCLUSION

Despite the undoubted effectiveness of chemotherapeutic treatment in GTN, problems related to toxicity of chemotherapy and chemo-resistant disease have led to reconsideration of the use of hysterectomy. In selected cases, a hysterectomy may be an effective treatment to either reduce or eliminate tumor bulk. Upon decision a number of drawbacks should however be considered.

Although complete remission following hysterectomy was incidentally reported in patients with metastatic disease, in general the procedure was less effective in patients with metastatic disease than in patients with localized disease. For patients with widespread metastatic disease however, the benefit of hysterectomy generally lies in the removal of chemotherapy-resistant tumor bulk to improve survival. Furthermore, due to prominent uterine vasculature, patients with GTN are at increased risk of life-threatening hemorrhage during hysterectomy [17] and initiation of chemotherapy may be delayed should postoperative complications occur [27]. Additionally, given the number of patients who need further chemotherapeutic treatment following hysterectomy, all patients still need to be monitored with serum hCG measurements during follow-up.

REFERENCES

1. Seckl MJ, Sebire NJ, Fisher RA, et al. Gestational trophoblastic disease: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24 Suppl 6**: vi39-50.
2. Lurain JR. Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. *Am J Obstet Gynecol* 2010; **203**(6): 531-9.
3. Froeling FE, Seckl MJ. Gestational trophoblastic tumours: an update for 2014. *Curr Oncol Rep* 2014; **16**(11): 408.
4. Brewer JI, Smith RT, Pratt GB. Choriocarcinoma. Absolute 5 year survival rates of 122 patients treated by hysterectomy. *Am J Obstet Gynecol* 1963; **85**: 841-3.
5. Feng F, Xiang Y, Li L, Wan X, Yang X. Clinical parameters predicting therapeutic response to surgical management in patients with chemotherapy-resistant gestational trophoblastic neoplasia. *Gynecol Oncol* 2009; **113**(3): 312-5.
6. Soper JT. Gestational trophoblastic disease. *Obstet Gynecol* 2006; **108**(1): 176-87.
7. Berkowitz RS, Goldstein DP. Current management of gestational trophoblastic diseases. *Gynecol Oncol* 2009; **112**(3): 654-62.
8. Papadopoulos AJ, Foskett M, Seckl MJ, et al. Twenty-five years' clinical experience with placental site trophoblastic tumors. *J Reprod Med* 2002; **47**(6): 460-4.
9. Hassadia A, Gillespie A, Tidy J, et al. Placental site trophoblastic tumour: clinical features and management. *Gynecol Oncol* 2005; **99**(3): 603-7.
10. Schmid P, Nagai Y, Agarwal R, et al. Prognostic markers and long-term outcome of placental-site trophoblastic tumours: a retrospective observational study. *Lancet* 2009; **374**(9683): 48-55.
11. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet* 2010; **376**(9742): 717-29.
12. Doumplis D, Al-Khatib K, Sieunarine K, et al. A review of the management by hysterectomy of 25 cases of gestational trophoblastic tumours from March 1993 to January 2006. *BJOG* 2007; **114**(9): 1168-71.
13. Doll KM, Soper JT. The role of surgery in the management of gestational trophoblastic neoplasia. *Obstet Gynecol Surv* 2013; **68**(7): 533-42.
14. Newlands ES, Holden L, Seckl MJ, McNeish I, Strickland S, Rustin GJ. Management of brain metastases in patients with high-risk gestational trophoblastic tumors. *J Reprod Med* 2002; **47**(6): 465-71.
15. Lurain JR, Nejad B. Secondary chemotherapy for high-risk gestational trophoblastic neoplasia. *Gynecol Oncol* 2005; **97**(2): 618-23.
16. Lurain JR. Gestational trophoblastic disease II: classification and management of gestational trophoblastic neoplasia. *Am J Obstet Gynecol* 2011; **204**(1): 11-8.
17. Hanna RK, Soper JT. The role of surgery and radiation therapy in the management of gestational trophoblastic disease. *Oncologist* 2010; **15**(6): 593-600.
18. Elias KM, Shoni M, Bernstein M, Goldstein DP, Berkowitz RS. Complete hydatidiform mole in women aged 40 to 49 years. *J Reprod Med* 2012; **57**(5-6): 254-8.
19. Yedema KA, Verheijen RH, Kenemans P, et al. Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. *Am J Obstet Gynecol* 1993; **168**(3 Pt 1): 787-92.

20. Thomas CM, Segers MF, Houx PC. Comparison of the analytical characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. *Ann Clin Biochem* 1985; **22 (Pt 3)**: 236-46.
21. Eysbouts YK, Massuger L, Thomas C, et al. Dutch Risk Classification and FIGO 2000 for Gestational Trophoblastic Neoplasia Compared. *Int J Gynecol Cancer* 2016.
22. Lok CA, Ansink AC, Grootfaam D, van der Velden J, Verheijen RH, ten Kate-Booij MJ. Treatment and prognosis of post term choriocarcinoma in The Netherlands. *Gynecol Oncol* 2006; **103**(2): 698-702.
23. van de Kaa CA, Schijf CP, de Wilde PC, Hanselaar AG, Vooijs PG. The role of deoxyribonucleic acid image cytometric and interphase cytogenetic analyses in the differential diagnosis, prognosis, and clinical follow-up of hydatidiform moles. A report from the Central Molar Registration in The Netherlands. *Am J Obstet Gynecol* 1997; **177**(5): 1219-29.
24. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivariate behavioral research* 2011; **46**(3): 399-424.
25. Clark RM, Nevadunsky NS, Ghosh S, Goldstein DP, Berkowitz RS. The evolving role of hysterectomy in gestational trophoblastic neoplasia at the New England Trophoblastic Disease Center. *J Reprod Med* 2010; **55**(5-6): 194-8.
26. Pires LV, Uberti EM, Fajardo Mdo C, et al. Role of hysterectomy in the management of patients with gestational trophoblastic neoplasia: importance of receiving treatment in reference centers. *J Reprod Med* 2012; **57**(7-8): 359-68.
27. Cagayan MS, Magallanes MS. The role of adjuvant surgery in the management of gestational trophoblastic neoplasia. *J Reprod Med* 2008; **53**(7): 513-8.
28. Ghaemmaghami F, Ashrafgangooei T, Gillani MM, Mosavi A, Behtash N. Major surgeries performed for gestational trophoblastic neoplasms in a teaching hospital in Tehran, Iran. *J Gynecol Oncol* 2011; **22**(2): 97-102.
29. El-Lamie IK, Shehata NA, Abou-Loz SK, Ei-Lamie KI. Experience of the Gynecologic Oncology Unit at Ain Shams University in the treatment of gestational trophoblastic tumors. *Int J Gynecol Cancer* 2000; **10**(6): 488-96.
30. Fulop V, Szigetvari I, Szepesi J, Vegh G, Zsirai L, Berkowitz RS. The Role of Surgery in the Management of Gestational Trophoblastic Neoplasia The Hungarian Experience. *J Reprod Med* 2016; **61**(5-6): 197-204.
31. Hammond CB, Weed JC, Jr., Currie JL. The role of operation in the current therapy of gestational trophoblastic disease. *Am J Obstet Gynecol* 1980; **136**(7): 844-58.
32. Jones WB, Lewis JL, Jr. Integration of surgery and other techniques in the management of trophoblastic malignancy. *Obstet Gynecol Clin North Am* 1988; **15**(3): 565-76.
33. Lurain JR, Singh DK, Schink JC. Role of surgery in the management of high-risk gestational trophoblastic neoplasia. *J Reprod Med* 2006; **51**(10): 773-6.
34. Fang J, Wang S, Han X, An R, Wang W, Xue Y. Role of adjuvant hysterectomy in management of high-risk gestational trophoblastic neoplasia. *Int J Gynecol Cancer* 2012; **22**(3): 509-14.
35. Suzuka K, Matsui H, Iitsuka Y, Yamazawa K, Seki K, Sekiya S. Adjuvant hysterectomy in low-risk gestational trophoblastic disease. *Obstet Gynecol* 2001; **97**(3): 431-4.
36. Lehman E, Gershenson DM, Burke TW, Levenback C, Silva EG, Morris M. Salvage surgery for chemorefractory gestational trophoblastic disease. *J Clin Oncol* 1994; **12**(12): 2737-42.

37. Pisal N, North C, Tidy J, Hancock B. Role of hysterectomy in management of gestational trophoblastic disease. *Gynecol Oncol* 2002; **87**(2): 190-2.
38. Bagshawe KD. Risk and prognostic factors in trophoblastic neoplasia. *Cancer* 1976; **38**(3): 1373-85.
39. Ngan HY, Bender H, Benedet JL, et al. Gestational trophoblastic neoplasia, FIGO 2000 staging and classification. *Int J Gynaecol Obstet* 2003; **83 Suppl 1**: 175-7.
40. Brown J, Naumann RW, Seckl MJ, Schink J. 15years of progress in gestational trophoblastic disease: Scoring, standardization, and salvage. *Gynecol Oncol* 2016.

Chapter 6

Dutch risk classification and FIGO 2000 for gestational trophoblastic neoplasia compared

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ABSTRACT

Objective: Over the years there has been a wide variety of classification systems in use worldwide to stratify patients between single versus multi-agent chemotherapy, hindering comparison of international research results. Present study presents a retrospective comparison of the FIGO 2000 and Dutch risk classification system for gestational trophoblastic neoplasia.

Methods: All patients diagnosed with GTN between January 2003 and December 2012 at the trophoblastic disease center in London were retrospectively scored according to the Dutch Classification system (n=813)

Results: An extensive overlap between both scoring systems was seen, even though items and relative value of items was quite distinct. The Dutch system seems to be simpler and easier to apply in all situation, a degree of overtreatment can however be presumed with the use of either system.

Conclusions: Whilst it is likely that outcome is indeed affected by the individual factors used in both systems, many factors relate to tumor bulk and may not be independently prognostic. We therefore believe that further refinement of the classification systems and their underlying prognostic items plus any new items that appear promising would be useful.

INTRODUCTION

Gestational trophoblastic disease (GTD) comprises a group of diseases, ranging from the pre-malignant partial (PHM) and complete hydatidiform moles (CHM) to malignant choriocarcinoma (CC), placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT) [1,2]. Nowadays GTN are among the most curable malignancies, with cure rates approaching 90%, even in the presence of metastases [2-5].

As clinical scoring systems predict the potential for resistance to single-agent chemotherapy with great accuracy, classification of GTN patients with recognized prognostic criteria and subsequent selection of appropriate treatment is now widely accepted [6-8]. Low-risk patients are often treated with methotrexate combined with folinic acid, with actinomycin D as an acceptable alternative in case of toxicity or resistance. High-risk patients should be treated with a multi-agent chemotherapy regimen. The EMA/CO protocol consisting of etoposide, methotrexate combined with folinic acid and actinomycin D alternating weekly with cyclophosphamide and vincristine has proven efficacy in primary treatment for high-risk patients [9-15]. Patients with PSTT are classified separately as first line treatment comprises hysterectomy due to relatively chemo resistant disease [16-19].

Due to wide variety of classification systems worldwide however, it has been difficult to evaluate patient care and perform a meaningful comparison of management outcomes [6,7,20,21]. It is likely that some patients have been either under- or overtreated, resulting in increased chemo resistance or elongation of treatment and treatment toxicity, respectively.

The prognostic scoring system Bagshawe suggested in 1976 considered different weights for various prognostic factors. It provided the basis for the WHO classification system, Charing Cross system and later the FIGO 2000 [8,22,23]. The FIGO 2000 is a revised staging and classification system comprising a number of prognostic factors as shown in Table 1. Patients with a score of 0-6 are considered low-risk for developing resistance to single-agent chemotherapy, a score of 7 or higher is considered high-risk [7,20,24]. The FIGO 2000 has gained wide acceptance worldwide and has been field tested in Sheffield UK [22].

Table 1. FIGO 2000 Classification system for GTN

Score	0	1	2	4
Age (years)	<40	≥40	-	-
Antecedent pregnancy	Mole	Abortion	Term	-
Interval from index pregnancy (months)	<4	4-6	7-12	≥13
Pre-treatment serum hCG (IU/L)	$<10^3$	$< 10^4$	$< 10^5$	$\geq 10^5$
Largest tumor size (cm)	<3	3-4	≥5	-
Site of metastases	Lung	Spleen, kidney	Gastro-intestinal	Liver, brain
Number of metastases	-	1-4	5-8	>8
Previous failed chemotherapy	-	-	Single drug	Multi drug

A total score of 0-6 = low-risk; score ≥ 7 = high-risk

In 1983, the Dutch Working Party on Trophoblastic Disease proposed a classification system using a number of absolute criteria for dividing patients into low- or high-risk for developing single-agent resistance, including antecedent term pregnancy and previous chemotherapeutic failure, shown in Table 2[8,25]. It relies on absolute divergence with the use of a confined number of criteria, resulting in a relatively straightforward system. The system is still employed today due to the use of a small set of easily retrievable factors and the assumption that serious prognostic factors such as liver involvement and interval are taken as absolute criteria for high-risk treatment [25].

Table 2. Dutch classification system for GTN

Risk group	Criteria
Low-risk	1. Antecedent pregnancy was a mole or miscarriage 2. Metastases confined to vagina or lungs 3. No previous failed chemotherapy 4. Interval from end of index pregnancy to treatment does not exceed 12 months
High-risk	1. Antecedent pregnancy was a term pregnancy 2. Metastases in more than one site (outside the uterus) 3. Metastases in one or more of the following organs: brain, liver, spleen, kidney, gastrointestinal tract 4. Failure of previous chemotherapy 5. Interval from end of index pregnancy to treatment exceeds 12 months

When one of the high-risk criteria is present, patients will be classified as high-risk and treated accordingly

To date, an appraisal of the Dutch classification system in comparison to the FIGO 2000 has not been performed. To validate and improve management of GTN however, it is essential to reach consensus on scoring systems and treatment protocols subsequently.

MATERIALS AND METHODS

Management protocols

In the United Kingdom, all patients diagnosed with trophoblastic disease are registered centrally for hCG monitoring and follow-up. All patients registered with the Charing Cross GTD service gave consent for this to occur as part of the registration process. Since 2000 a division in low- and high-risk patients is made in accordance with the FIGO 2000 classification system for GTN. This determined the given treatment, with low-risk patients being treated with methotrexate and high-risk patients being treated with multi-agent chemotherapy according to the EMA/CO regimen. In selected cases, actinomycin D is used when toxicity or resistance to methotrexate is present [1]. In patients with detected pulmonary metastases intrathecal methotrexate as central nervous system (CNS) prophylaxis was additionally administered [26].

In the Netherlands patients with GTD are assigned to a low- or high-risk group, according to the Dutch classification system for GTN. All patients with GTN are discussed in the Dutch Working party on Trophoblastic Disease and high-risk patients are treated in referral hospitals. Treatment for low- and high-risk patients respectively is fairly similar to the British regimen, mainly involving methotrexate for low-risk groups, with actinomycin D as an appropriate alternative and EMA/CO for high-risk groups [27].

Patient selection

The electronic database of the trophoblastic disease center at Charing Cross Hospital in London was screened to identify all patients with GTN diagnosed between January 2003 and December 2012. All selected patients were retrospectively scored according to the Dutch Classification system for GTN and an overview of patients characteristics, treatment course and outcome was composed.

One patient with PSTT was excluded because the classification and treatment for these patients is different. Furthermore, 15 patients were excluded when all or some criteria required for risk classification in either FIGO or Dutch classification were missing.

Statistics

A computerized database was developed, descriptive analyses was carried out with SPSS for windows 20.0.

RESULTS

Patient distribution

A total of 828 patients were treated with chemotherapy for GTD in the UK between 2003 and 2012, of which 813 patients had sufficient data available from the database for further analyses. Table 3 summarizes the distribution of patients according to the FIGO 2000 and Dutch classification system. In terms of risk classification both scoring systems were equivalent in 93.4% of cases. Discordant risk distribution when applying

Table 3. Distribution of patients in each subgroup according to FIGO 2000 and Dutch classification system.

	Low-risk FIGO	High-risk FIGO	Total
Low-risk Dutch system	711 (87.5%)	39 (4.8%)	**750 (92.2%)**
High-risk Dutch system	14 (1.7%)	49 (6.0%)	**63 (7.8%)**
Total	**725 (89.2%)**	**88 (10.8%)**	

the FIGO classification system and Dutch classification system was seen in 53 patients (6.5%). In contrast with the FIGO 2000, fourteen patients (1.7%) would have been commenced on high risk treatment. Thirty-nine patients (4.8%) would have been low-risk according to the Dutch classification and therefore treated with methotrexate instead of EMA/CO.

All patients with widespread metastases and previous chemotherapeutic treatment were considered high-risk in both FIGO 2000 and Dutch classification system. One death associated with acute renal failure occurred as a result of complications during therapy after multi-agent treatment for widespread disease with metastases in both lungs and liver. The categorization for this patient by either classification system was high-risk.

Patient characteristics and discrepancies

Of the fourteen patients who would have been high-risk by the Dutch classification (but low by the FIGO 2000 criteria), one patient erroneously received EMA/CO as

first line treatment. The other thirteen patients were commenced on single-agent treatment, with six achieving complete and sustained remission on single-agent and seven developing resistance to methotrexate and requiring subsequent EMA/CO. The antecedent pregnancy was a term gestation in 12 of these patients which made them high-risk in the Dutch classification system but their median FIGO score was 4 (range of 2 to 6). Patient characteristics and their decisive FIGO criteria per risk classification group are shown in Table 4.

Table 4. Patient characteristics and decisive FIGO criteria per risk classification group

	FIGO low-risk		**FIGO high-hisk**	
	Dutch low-risk N=711	**Dutch high-risk** N=14	**Dutch low-risk** N=39	**Dutch high-risk** N=49
Age >40 years	135 (19%)	-	24 (62%)	8 (16%)
Widespread metastases [a]	-	-	-	15 (37%)
Largest tumour >5cm	151 (22%)	1 (7%)	34 (87%)	22 (54%)
>8 metastases	-	-	-	20 (49%)
Term pregnancy	-	13 (87%)	-	48 (98%)
Previous failed chemotherapy	-	-	-	5 (12%)
hCG pretreatment >100.000 IU/l	42 (6%)	13 (87%)	35 (90%)	17 (43%)
Median FIGO score *(Min- Max)*	3 (0-6)	4 (2-6)	7 (7-12)	12 (7-23)

[a] Kidney, Spleen, Gastro-intestinal, Liver and/or Brain

Conversely 39 patients who would have been classified as low-risk by the Dutch classification were high-risk by the FIGO 2000 criteria. The majority of these patients (90%) had a serum hCG value preceding treatment of more than 100.000 IU/l and tumor size of 5 cm, resulting in a combined score on the FIGO 2000 of 6 points. Moreover, the number of patients with an age of over 40 was substantially higher in this group, resulting in the additional 1 point on the FIGO 2000 for age in 62% of cases. Median FIGO score in this group was calculated at 7 (range of 7 to 12), whereas 94% of these patients had FIGO score of 7 or 8. In comparison, a median FIGO score of 12 was seen in the group considered high-risk by either classification system, with a FIGO score of 7 or 8 in 28% of cases. Complete sustained remission was achieved in all these patients with multi-agent chemotherapy with a median duration of treatment to normalization of 3 months (minimum 1, maximum 6 months).

DISCUSSION

In the present study a retrospective comparison of the FIGO 2000 and Dutch classification system for patients with GTN was performed. Both systems turned out to be widely equivalent with similar risk classification in up to 93.4% of cases, whereas the one death was only associated with high risk scores in both systems. Discordant risk scores when applying both systems were seen in 53 patients (6.5%), of which 14 patients (1.7%) would have been considered high-risk according to the Dutch system, predominantly due to antecedent term pregnancy. Six of these patients achieved complete remission with MTX, whereas the remaining eight patients were fully salvaged with second line EMA/CO. It is important to notice that even though numbers are small, overtreatment of these patients is of great clinical relevance. Combination therapy with EMA/CO as is recommended in case of high-risk disease is certainly toxic causing alopecia in nearly all, myelosuppression in most and neuropathy in many patients. Whilst these side-effects are all reversible, they are unpleasant and in the long-term the treatment advances the menopause date by about 3 years [5]. In contrast, significant toxicity to the MTX/FA therapy used in the UK and the Netherlands in the form of severe stomatitis or serositis is very uncommon and there are no long term toxicities. The subsequent cancer risk for patients cured of GTN with modern chemotherapy regimen now appears similar to the general population, provided treatment duration is kept under 6 months [28].

So what about the 39 (4.8%) patients considered as high-risk according to the FIGO 2000 but low-risk if the Dutch system had been employed? This implies a potential for undertreatment when the Dutch classification system would have been used. Since this is a retrospective study, all of these individuals had already been treated with EMA/CO. Consequently, their possible response to single-agent MTX cannot be evaluated. The higher total FIGO scores for patients considered high-risk by both systems in comparison to the patients considered high-risk by FIGO but low-risk by the Dutch classification however illustrate existing differences in prognostic factors and thus possible differences in severity of disease. It is therefore conceivable that some patients in this group may have been responsive to MTX/FA treatment alone and that the more aggressive multi-agent regimen with its significant toxicity could have been avoided. A prospective designed trial rather than the present retrospective analysis would be needed to assess this particular patient group. Furthermore, a ret-

rospective assignment of FIGO 2000 classification on the Dutch patient cohort would enable further insight in underlying differences. The treatment course of patients assigned to low-risk treatment in the Netherlands, when high-risk treatment would have been recommended according to the FIGO 2000 might demonstrate patients overtreated by this system. Unfortunately the individual prognostic scores on tumor size and number of metastases as requested in the FIGO 2000 were generally not available. Therefore retrospective evaluation of FIGO classification was not possible in this cohort. A comparison of the proportion of misclassified patients according to both systems is therefore unavailable.

Patients with a score of 6 or below in the FIGO system and term antecedent pregnancy may still present a gray zone between true low and high-risk disease. Patients can however be safely commenced on low-risk treatment with resistance being easily salvaged with EMA/CO without compromising mortality as this is only associated with higher FIGO scores [22,23,29]. Serious prognostic factors such as extended metastatic disease and interval between antecedent pregnancy and subsequent GTN receive only relative weight in the FIGO 2000, where the Dutch system takes such factors as absolute decisive criteria. All patients with an interval of over 12 months or extended metastatic disease however were classified as high-risk using the FIGO 2000 system, implying that the involved tumor burden is probably simultaneously recognized by presence of other criteria. An intrinsic correlation between term pregnancy, metastatic disease and high tumor load might also reveal whether term pregnancy is associated with higher resistance and mortality rates. In this regard, the risk criteria involved in the FIGO 2000 have previously been assessed in multivariate analyses, to identify the most significant predictors of treatment failure and poor prognosis. Most studies were however conducted in the eighties and early nineties, when treatment selection criteria, treatment regimens and outcome varied widely [30-33]. Therefore, whilst these studies re-emphasized the problems of comparing worldwide data sets, it is unclear how valid the findings are today.

In conclusion, the present study has highlighted the extensive overlap between both FIGO and Dutch scoring/staging systems even though items and relative value of items was quite distinct. It is not immediately obvious from the results of this study if one system is clearly superior to the other. The Dutch Classification seems to be simpler and easier to apply in all situations. However in selected cases the Dutch system

actually leads to an overtreatment of patients. Unfortunately, since we did not have all the FIGO data recorded in the Dutch patients, it has not been possible for us to determine the potential rate of overtreatment with multi-agent chemotherapy in the FIGO 2000 scoring system. A degree of overtreatment with use of the FIGO system can however be presumed as well considering the present differences in total FIGO score for high-risk FIGO patients with either low-risk of high-risk Dutch classification. If the overtreatment rate was lower in the FIGO scoring system this would provide a strong case to replace the Dutch classification system. Pending this data, the only case to replace the Dutch system with the FIGO system is that of international unification to enable better cross correlation of therapy results between nations. In the meantime, we believe that further refinement of our risk classification systems through fresh univariate and multivariate analysis of the FIGO prognostic variables plus any new factors that appear promising would be useful.

REFERENCES

1. Seckl MJ, Sebire NJ, Fisher RA, et al. Gestational trophoblastic disease: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24 Suppl 6**: vi39-50.
2. Lurain JR. Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. *Am J Obstet Gynecol* 2010; **203**(6): 531-9.
3. Soper JT. Gestational trophoblastic disease. *Obstet Gynecol* 2006; **108**(1): 176-87.
4. Berkowitz RS, Goldstein DP. Current management of gestational trophoblastic diseases. *Gynecol Oncol* 2009; **112**(3): 654-62.
5. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet* 2010; **376**(9742): 717-29.
6. Bagshawe KD. Risk and prognostic factors in trophoblastic neoplasia. *Cancer* 1976; **38**(3): 1373-85.
7. Ngan HY, Bender H, Benedet JL, et al. Gestational trophoblastic neoplasia, FIGO 2000 staging and classification. *Int J Gynaecol Obstet* 2003; **83 Suppl 1**: 175-7.
8. Hancock BW. Staging and classification of gestational trophoblastic disease. *Best Pract Res Clin Obstet Gynaecol* 2003; **17**(6): 869-83.
9. Bower M, Newlands ES, Holden L, et al. EMA/CO for high-risk gestational trophoblastic tumors: results from a cohort of 272 patients. *J Clin Oncol* 1997; **15**(7): 2636-43.
10. Matsui H, Suzuka K, Iitsuka Y, Seki K, Sekiya S. Combination chemotherapy with methotrexate, etoposide, and actinomycin D for high-risk gestational trophoblastic tumors. *Gynecol Oncol* 2000; **78**(1): 28-31.
11. Escobar PF, Lurain JR, Singh DK, Bozorgi K, Fishman DA. Treatment of high-risk gestational trophoblastic neoplasia with etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine chemotherapy. *Gynecol Oncol* 2003; **91**(3): 552-7.
12. Lurain JR, Singh DK, Schink JC. Primary treatment of metastatic high-risk gestational trophoblastic neoplasia with EMA-CO chemotherapy. *J Reprod Med* 2006; **51**(10): 767-72.
13. Turan T, Karacay O, Tulunay G, et al. Results with EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine) chemotherapy in gestational trophoblastic neoplasia. *Int J Gynecol Cancer* 2006; **16**(3): 1432-8.
14. Lu WG, Ye F, Shen YM, et al. EMA-CO chemotherapy for high-risk gestational trophoblastic neoplasia: a clinical analysis of 54 patients. *Int J Gynecol Cancer* 2008; **18**(2): 357-62.
15. Alifrangis C, Agarwal R, Short D, et al. EMA/CO for high-risk gestational trophoblastic neoplasia: good outcomes with induction low-dose etoposide-cisplatin and genetic analysis. *J Clin Oncol* 2013; **31**(2): 280-6.
16. Papadopoulos AJ, Foskett M, Seckl MJ, et al. Twenty-five years' clinical experience with placental site trophoblastic tumors. *J Reprod Med* 2002; **47**(6): 460-4.
17. Hassadia A, Gillespie A, Tidy J, et al. Placental site trophoblastic tumour: clinical features and management. *Gynecol Oncol* 2005; **99**(3): 603-7.
18. Schmid P, Nagai Y, Agarwal R, et al. Prognostic markers and long-term outcome of placental-site trophoblastic tumours: a retrospective observational study. *Lancet* 2009; **374**(9683): 48-55.
19. Lurain JR. Gestational trophoblastic disease II: classification and management of gestational trophoblastic neoplasia. *Am J Obstet Gynecol* 2011; **204**(1): 11-8.

20. Kohorn EI, Goldstein DP, Hancock BW, et al. Workshop Report: Combining the staging system of the International Federation of Gynecology and Obstetrics with the scoring system of the World Heath Organization for Trophoblastic Neoplasia. Report of the Working Committee of the International Society for the Study of Trophoblastic Disease and the International Gynecologic Cancer Society. *Int J Gynecol Cancer* 2000; **10**(1): 84-8.
21. Goldstein DP, Zanten-Przybysz IV, Bernstein MR, Berkowitz RS. Revised FIGO staging system for gestational trophoblastic tumors. Recommendations regarding therapy. *J Reprod Med* 1998; **43**(1): 37-43.
22. Hancock BW, Welch EM, Gillespie AM, Newlands ES. A retrospective comparison of current and proposed staging and scoring systems for persistent gestational trophoblastic disease. *Int J Gynecol Cancer* 2000; **10**(4): 318-22.
23. El-Helw LM, Coleman RE, Everard JE, et al. Impact of the revised FIGO/WHO system on the management of patients with gestational trophoblastic neoplasia. *Gynecol Oncol* 2009; **113**(3): 306-11.
24. Committee FO. FIGO staging for gestational trophoblastic neoplasia 2000. FIGO Oncology Committee. *Int J Gynaecol Obstet* 2002; **77**(3): 285-7.
25. Dijkema HE, Aalders JG, de Bruijn HW, Laurini RN, Willemse PH. Risk factors in gestational trophoblastic disease, and consequences for primary treatment. *Eur J Obstet Gynecol Reprod Biol* 1986; **22**(3): 145-52.
26. Sita-Lumsden A, Short D, Lindsay I, et al. Treatment outcomes for 618 women with gestational trophoblastic tumours following a molar pregnancy at the Charing Cross Hospital, 2000-2009. *Br J Cancer* 2012; **107**(11): 1810-4.
27. Lybol C, Sweep FC, Harvey R, et al. Relapse rates after two versus three consolidation courses of methotrexate in the treatment of low-risk gestational trophoblastic neoplasia. *Gynecol Oncol* 2012; **125**(3): 576-9.
28. Savage P, Cooke R, O'Nions J, et al. Effects of single-agent and combination chemotherapy for gestational trophoblastic tumors on risks of second malignancy and early menopause. *J Clin Oncol* 2015; **33**(5): 472-8.
29. Kohorn EI. The trophoblastic Tower of Babel: classification systems for metastatic gestational trophoblastic neoplasia. *Gynecol Oncol* 1995; **56**(2): 280-8.
30. Soper JT, Evans AC, Conaway MR, Clarke-Pearson DL, Berchuck A, Hammond CB. Evaluation of prognostic factors and staging in gestational trophoblastic tumor. *Obstet Gynecol* 1994; **84**(6): 969-73.
31. Azab MB, Pejovic MH, Theodore C, et al. Prognostic factors in gestational trophoblastic tumors. A multivariate analysis. *Cancer* 1988; **62**(3): 585-92.
32. Lurain JR, Casanova LA, Miller DS, Rademaker AW. Prognostic factors in gestational trophoblastic tumors: a proposed new scoring system based on multivariate analysis. *Am J Obstet Gynecol* 1991; **164**(2): 611-6.
33. Kim SJ, Bae SN, Kim JH, et al. Effects of multiagent chemotherapy and independent risk factors in the treatment of high-risk GTT--25 years experiences of KRI-TRD. *Int J Gynaecol Obstet* 1998; **60 Suppl 1**: S85-96.

Chapter 7

Can the FIGO 2000 scoring system for gestational trophoblastic neoplasia be simplified? A new retrospective analysis from a nation-wide data-set

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ABSTRACT

Background: Worldwide introduction of the FIGO 2000 scoring system has provided an effective means to stratify patients with gestational trophoblastic neoplasia (GTN) to single- or multi-agent chemotherapy. However, the system is quite elaborate with an extensive set of risk factors. In this study, we re-evaluate all prognostic risk factors involved in the FIGO 2000 scoring system and examine if simplification is feasible.

Patients and methods: Between January 2003 and December 2012, 813 patients diagnosed with GTN were identified at the Trophoblastic Disease Center in London and scored using the FIGO 2000. Multivariable analysis and stepwise logistic regression were carried out to evaluate if the FIGO 2000 scoring system could be simplified.

Results: Of the eight FIGO risk factors only pre-treatment serum human chorionic gonadotropin (hCG) levels exceeding 10,000 IU/l (OR = 5.0; CI 2.5-10.4) and 100,000 IU/l (OR = 14.3; CI 4.7-44.1), interval exceeding 7 months since antecedent pregnancy (OR = 4.1; CI 1.0-16.2) and tumor size of over 5 cm (OR = 2.2; CI 1.3-3.6) were identified as independently predictive for single-agent resistance. In addition, increased risk was apparent for antecedent term pregnancy (OR = 3.4; CI 0.9-12.7) and the presence of 5 or more metastases (OR = 3.5; CI 0.4-30.4), but patient numbers in these categories were relatively small. Stepwise logistic regression identified a simplified risk scoring model comprising age, pre-treatment serum hCG, number of metastases, antecedent pregnancy and interval but omitting tumor size, previous failed chemotherapy and site of metastases. With this model only 1 of 725 patients was classified differently from the FIGO 2000 system.

Conclusion: Our simplified alternative using only five of the FIGO prognostic factors appears to be an accurate system for discriminating patients requiring single as opposed to multi-agent chemotherapy. Further work is urgently needed to validate these findings.

INTRODUCTION

Gestational trophoblastic disease (GTD) comprises a group of pregnancy related disorders including the premalignant complete and partial hydatidiform moles through to the malignant invasive mole, choriocarcinoma, placental site trophoblastic tumor (PSTT), and epithelioid trophoblastic tumor (ETT) [1]. The malignant counterparts are often collectively referred to as gestational trophoblastic neoplasia (GTN). Fortunately, with the introduction of effective chemotherapy, GTN has become highly curable with overall survival rates approaching 90% [1-4].

Cure for non-PSTT/ETT forms of GTN can often be achieved with single-agent chemotherapy comprising either methotrexate (with or without folinic acid rescue) or actinomycin D. However some patients require multi-agent chemotherapy most commonly comprising etoposide, methotrexate, actinomycin D alternating weekly with cyclophosphamide and vincristine (EMA/CO) to achieve long-term remission [4]. Over the years, several important predictors of unfavorable prognosis such as serum human chorionic gonadotropin (hCG) levels and site of metastases have been proposed [2,5,6] to stratify patients between single or multi-agent therapies. These factors have formed the basis of a number of different clinical scoring systems [5,7], used to distinguish GTN patients as either having a low or high-risk of developing resistance to single-agent chemotherapy.

To help facilitate comparison of datasets between international treatment centers, a renewed scoring system was introduced in 2000 [8,9]. The new FIGO 2000 risk scoring system was based on a combination of anatomic and pathophysiological features of the disease and was developed with the effort of a number of international societies including the International Society for the Study of Trophoblastic Diseases (ISSTD), the International Gynaecologic Cancer Society (IGCS) and International Fedaration of Gynaecology and Obstetrics (FIGO)[9,10].

The worldwide introduction of the FIGO 2000 has provided an opportunity to reach agreement on classification and subsequent treatment for patients with GTN. However, the system is quite elaborate and comprises an extensive set of risk factors, several of which relate to tumor bulk and may therefore not be independently prognostic [2,6,11]. A greater number of factors involved will likely result in an increased

variability in scoring and classification. Especially in a low-incidence disease like GTN, global unification is essential to optimize management.

In this study, fifteen years following the introduction of FIGO 2000, we decided to re-evaluate all prognostic factors involved in the FIGO 2000 scoring system to determine whether simplification of this system is feasible.

MATERIALS AND METHODS

Patients

All patients diagnosed with GTN between January 2003 and December 2012 were identified from the electronic database of the Trophoblastic Disease Center at Charing Cross Hospital in London. Patients with a histopathological diagnosis of PSTT or ETT were excluded, resulting in 813 GTN patients of which 725 were low-risk and 88 were high-risk by FIGO 2000 scoring. Uni- and multi-variable analyses were conducted for 705 of 725 low-risk patients, since this was the total number of cases where their response to single-agent therapy was known. The remaining 20 patients had FIGO score 6 disease and either wanted high-risk treatment or were advised to start high-risk treatment because of a very high pre-treatment serum hCG typically in excess of 400,000 IU/L [12].

Management protocols

Prior to treatment all patients were assigned to low or high-risk groups in accordance with the FIGO 2000 scoring system for GTN (Supplementary Table 1). Low-risk patients received single-agent methotrexate with folinic acid rescue (50 mg intramuscular MTX on days 1, 3, 5, and 7 and folinic acid 15 mg orally on days 2, 4, 6, and 8). In patients developing resistance or unmanageable toxicity, therapy was changed to either single-agent actinomycin D (ActD) or multi-agent chemotherapy comprising etoposide, methotrexate and actinomycin D alternating weekly with cyclophosphamide and vincristine (EMA/CO). The decision to use ActD as opposed to EMA/CO was based on the serum hCG level at the point of resistance. Patients with an hCG ≤ 300 IU/L received ActD whilst those > 300 IU/L were given EMA/CO as previously described [13]. ActD was given as 0.5 mg intravenously on days 1-5 every two weeks [13]. Patients with disease resistant to ActD received EMA/CO chemotherapy subsequently. High-

risk patients received multi-agent chemotherapy with EMA/CO as first-line therapy. In patients presenting with very advanced disease, induction low-dose etoposide and cisplatin was given prior to commencing either EMA/CO or EP/EMA (etoposide and cisplatin alternating weekly with etoposide, methotrexate and actinomycin D). Appropriate adaptation for occult or overt CNS disease was provided as previously described [14,15]. Disease response and resistance to therapy was assessed by serum hCG measurements undertaken twice weekly until hCG was normal and then weekly until 6 weeks after completion of chemotherapy using the Charing Cross hCG radioimmunoassay as previously described [4].

Statistical analysis

The predictive value of the prognostic factors for chemoresistance to MTX or ActD was assessed in low-risk patients using univariate and multivariable logistic regression. Thereafter, a backward stepwise (Wald) logistic regression was carried out for all patients to evaluate if simplification of the original FIGO system was feasible. To minimize the number of low-risk patients unnecessary subjected to the more aggressive multi-agent chemotherapy with consequent toxicity, simplified models were only considered if at least 98% of patients had concordant FIGO classification. Guided by the previous results, a small set of modified FIGO models that best resembled classification of the original FIGO 2000 was constructed. Finally, with receiver operating characteristic (ROC) curves, the discriminating power of the alternative models in comparison to the original FIGO classification was evaluated. All statistical analyses were performed with SPSS for windows 22.0.

RESULTS

Patient characteristics of the 813 patients with GTN are shown in Table 1. Twenty-eight percent of low-risk patients eventually needed salvage multi-agent chemotherapy after initial MTX/FA with or without subsequent ActD. One death associated with acute renal failure occurred as a result of complications during multi-agent therapy for widespread disease.

Table 1. Patient characteristics [a]

Patient characteristic	Low-risk patients (N=725)		High-risk patients (N=88)	
	Mean (sd)	min-max	Mean (sd)	min-max
Age (years)	32.2 (8.0)	14-56	34.7 (9.2)	15-62
Pre-treatment ^{10}log serum hCG (IU/l)	3.8 (1.1)	1-7	4.9 (1.1)	2-7
Interval (months)	1.8 (2.1)	0-35	10.5 (33.2)	0-242
Duration of treatment (months) [b]	1.9 (2.2)	0-15	2.8 (1.6)	0-10
FIGO score	2.7 (1.6)	0-6	10.3 (3.9)	7-23
Antecedent pregnancy	Number	Percentage	Number	Percentage
Hydatidiform Mole	696	96.0 %	36	40.9 %
Miscarriage	17	2.3 %	4	4.5 %
Term	12	1.7 %	48	54.5 %
Tumor size (cm)				
<3cm	304	41.9 %	11	13.7 %
3-5cm	240	33.1 %	13	16.3 %
>5cm	152	21.0 %	56	70.0 %
Site of metastases				
Vagina	4	0.6 %	4	5.0 %
Lung	56	7.7 %	46	57.5 %
Liver	-	-	6	7.5 %
Brain	-	-	12	15.0 %
Other	-	-	5	6.3 %
Number of metastases				
None	662	91.4 %	23	28.7 %
1-4	58	8.0 %	23	28.7 %
5-8	4	0.6 %	7	8.8 %
>8	-	-	27	33.8 %

[a] For some patients scoring on one or more of the FIGO criteria was unavailable

[b] Duration of treatment is defined in months until normalization in serum hCG levels was reached

Table 2 shows the results of the univariate and multivariable analysis performed for low-risk patients treated with single-agent chemotherapy. Site of metastases and previous failed chemotherapy were not included in the analysis as all patients with widespread metastases or previous failed chemotherapy were classified as high-risk patients and therefore not treated with single-agent therapy. Tumor size, antecedent term pregnancy, interval and pre-treatment serum hCG were significant predictors for single-agent resistance in univariate analysis. In multivariable analysis, pre-treatment serum hCG levels exceeding 10,000 IU/L (OR = 5.0; CI 2.5-10.4) and 100,000 IU/L (OR = 14.3; CI 4.7-44.1), interval exceeding 7 months since antecedent pregnancy (OR = 4.1; CI 1.0-16.2) and tumor size of over 5 cm (OR = 2.2; CI 1.3-3.6) were all identified as independent predictive factors for resistance to single-agent therapy. An increased

Table 2. Univariate and multivariable analysis of prognostic factors for single-agent resistance

Variable	Rate of single-agent resistance (%)	Odds Ratio (95% CI)[a]	Odds Ratio (95% CI)[a]
		Univariate	Multivariable
Age (years)			
<40	159/572 (27.8%)		
≥40	34/133 (25.6%)	0.9 (0.6-1.4)	0.9 (0.6-1.5)
Antecedent pregnancy			
Hydatidiform Mole	183/677 (27.0%)		
Miscarriage	4/17 (23.5%)	0.8 (0.3-2.6)	0.6 (0.1-2.4)
Term	6/11 (54.5%)	3.2 (1.0-10.7)[b]	3.4 (0.9-12.7)
Interval (months)			
<4	179/617 (29.0%)		
4-6	9/73 (12.3%)	0.3 (0.2-0.7)[b]	1.1 (0.5-2.7)
7-12	5/14 (35.7%)	1.4 (0.4-4.1)	4.1 (1.0-16.2)[b]
>12	0/1 (0%)	-	-
Pre-treatment serum hCG (IU/l)			
<1000	19/167 (11.4%)		
1000-10.000	28/187 (15%)	1.4 (0.7-2.6)	1.6 (0.8-3.5)
10.000-100.000	127/324 (39.2%)	5.0 (3.0-8.5)[b]	5.0 (2.5-10.4)[b]
>100.000	19/27 (70.4%)	18.5 (7.1-48.0)[b]	14.3 (4.7-44.1)[b]
Tumor size (cm)			
<3cm	55/302 (18.2%)		
3-5cm	61/232 (26.3%)	1.6 (1.1-2.4)[b]	0.9 (0.6-1.4)
≥5cm	71/142 (50.0%)	4.5 (2.9-7.0)[b]	2.2 (1.3-3.6)[b]
Number of metastases			
None	172/644 (26.7%)		
1-4	19/56 (33.9%)	1.4 (0.8-2.5)	1.4 (0.7-2.6)
5-8	2/4 (50.0%)	2.7 (0.4-19.6)	3.5 (0.4-30.4)
>8	0/0 (0%)	-	-

[a] CI: Confidence Interval

[b] $p<0.05$

risk was apparent for antecedent term pregnancy (OR = 3.4; CI 0.9-12.7) and the presence of 5 or more metastases (OR = 3.5; CI 0.4-30.4). However, numbers in these categories were relatively small.

Using stepwise backwards (Wald) logistic regression, FIGO criteria lacking significant independent value were eliminated, identifying three simplified models. In these models 4 (model 2) or 5 (model 1 and 3) of the original 8 FIGO criteria were sufficient for identical risk classification in 99% of patients (Table 3)**.** The discriminating power of these simplified FIGO scoring systems was compared to the original FIGO 2000

Table 3. Alternative scoring systems and their performance with FIGO 2000 compared[a]

Model	AUC[b]	True Positive[c]	True Negative[c]	False Positive[c]	False Negative[c]	Sensitivity	Specitivity	Identical classification
Original FIGO 2000	1.000	694	73	0	0	1.00	1.00	100%
Model 1 Age Antecedent pregnancy Pre-treatment serum hCG Tumor size Number of metastases	0.999	693	70	2 [1,2 c]	4 [5,6,7,8 c]	0.99	0.97	99.1%
Model 2 Age Antecedent pregnancy Pre-treatment serum hCG Number of metastases	0.998	720	71	4 [1,2,3,4 c]	3 [5,7,8 c]	1.00	0.95	99.2%
Model 3 Age Antecedent pregnancy Interval Pre-treatment serum hCG Number of metastases	1.000	722	73	1 [4 b]	0	1.00	0.99	99.9%

[a] Allowing for the fact that the FIGO 2000 was already used in this particular data, the AUC, sensitivity and specificity for the original FIGO 2000 were consequently calculated at 1.0.

[b] Risk classification according to the FIGO 2000 was considered 'gold standard'

[c] For every alternative scoring system the number of discordant patients and corresponding case-numbers are highlighted

using ROC analysis. In model 1 and 2, six and seven patients respectively were classified differently. In model 3, with the elimination of tumor size, site of metastases and previous failed chemotherapy, classification for one patient changed from low-risk to high-risk. None of these patients had more than 4 metastases or metastases outside the lungs. Supplementary Table 2 shows the characteristics of all eight cases with a different risk classification when using one of the simplified alternatives in comparison to the FIGO 2000.

DISCUSSION

The FIGO 2000 comprises a weighted prognostic scoring system resulting in a calculated total score and subsequent classification of GTN patients with low-risk and high-risk of resistance to single-agent chemotherapy. Most prognostic factors relate to tumor bulk, it is therefore questionable whether all these factors are required for adequate classification of patients [16]. Furthermore, with the use of interrelated factors the actual weight for certain items could be overrepresented using FIGO 2000.

With use of uni- and multivariable logistic regression, a smaller selection of risk factors could be identified as significant predictors for single-agent resistance [1-4]. In concordance with other studies, both tumor size and pre-treatment serum hCG emerge as important prognostic variables in our analysis [5,11]. As all patients with GTN likely undergo imaging with pelvic ultrasound, tumor size can be derived quite easily in a non-invasive manner. In some cases, the volume of a trophoblastic tumor however may not represent the proportion of viable cells due to variations in the extent of necrosis and hemorrhage [17]. Serum hCG is a disease-specific tumor marker, associated with burden of disease and is easily measured quantitatively. hCG levels of over 10,000 IU/L and 100,000 IU/L in particular reflect strong relations to treatment failure in low-risk patients. As commercially available assays for quantification of serum hCG concentrations use different sets of antibodies and often a different standard, assay results strongly depend on the type of assay used. Although the effect is probably modest with high hCG levels, problems may occur with monitoring of response and follow-up in the lower range of hCG levels [1,17,18].

While antecedent term pregnancy and interval since diagnosis have been associated with poor prognosis in univariate analyses, they however lose their significant prognostic value in some multivariable analyses [2,6,19]. For interval, the resulting hazard ratio appears non-linear and results likely depend on the chosen cutoff time. A sensible cutoff point will probably be beyond 12 months since diagnosis, as suggested by Powles et al. [20]. In our study only few patients had an interval exceeding 7 months, and likewise an increased risk of single-agent resistance was seen. In patients with antecedent term pregnancies, we observed an increased risk of single-agent resistance, but even in this rather large cohort of patients, numbers in this subcategory remain small. Although choriocarcinoma could be considered a surrogate marker for antecedent term pregnancy, the latter term is preferred as histological confirmation is not always available. Problems with correct identification of the antecedent pregnancy and interval subsequently, can particularly occur when a patient has previously experienced an abortion without histological examination.

The effect of advanced age in GTD incidence has been evaluated regularly [21-23]. It's possible effect on the development of GTN and survival however has been under debate[2,5,6,11]. In line with the majority of studies, age was not identified as an independent prognostic factor in the present study. However, treatment often differs with advanced age, since hysterectomy is a reasonable treatment option when fertility preservation is not desired and a reduction of toxicity from chemotherapeutic regimens may be profitable. Furthermore, considering all factors required for staging, age is probably one with the least possible uncertainty [17].

For both site of metastases and number of metastases, measurements are highly dependent on the used imaging technology used. For practical purposes and uniformity, simple investigation tools such as X-ray provide adequate clinical guidance [17]. Only few patients with a high number of metastases (5 or more) exist, possible implications on prognosis therefore remain unclear. Furthermore, there is wide consensus on the effects of widespread metastases on single-agent resistance and survival [2,5,6,11]. In this cohort however, patients with widespread metastases were all characterized by a total FIGO score of over 10. Simultaneous presence of other prognostic factors has obviated the occurrence of misclassification in this group.

We however have to keep in mind that the present FIGO score, whilst only designed for stratifying patients between low and high-risk treatments is also used to identify patients at greatest risk of early death within 4 weeks of commencing therapy and late death from multi-drug resistant disease. These ultra-high risk patients, present with widespread metastatic disease, reflected by a very high FIGO score (>12), are at significant risk for pulmonary, intra-peritoneal or intracranial hemorrhage and may benefit from low-dose induction chemotherapy. Furthermore, those with liver metastases with or without brain metastases are at increased risk of late death [15]. Removal of criteria that reflect these factors in a simplified system would hinder identification of these patient groups [14]. Consequently, the new system will need to be carefully evaluated with sufficient patient numbers in the high- and ultra-high risk groups.

Consensus exists on the concept of re-staging in case of relapse with full re-assessment of spread of disease and previous chemotherapy response. Failure to respond to single-agent therapy already justifies the start of a different single-agent regimen or multi-agent therapy depending on hCG value. Confusion may however exist on the definition of failed chemotherapy. It would therefore be helpful to provide a clear definition on failed chemotherapy with the revised FIGO 2000 (i.e. rise of serum hCG after two chemotherapy cycles.

It appears that only a small proportion of FIGO 2000 prognostic factors is needed to differentiate patients with low versus high-risk of single-agent resistance. This could lead to a relatively straightforward system with a small subset of easily retrievable factors, ideally reducing variability in scoring and improving agreement between centers. A simplified model with age, pre-treatment serum hCG levels, number of metastases, antecedent pregnancy and interval alone resulted in an identical risk classification as the original FIGO 2000 in all but 1 of the 194 low-risk patients that needed to switch to high-risk therapy. Tumor size, previous failed chemotherapy and site of metastases did not provide much added value.

After fifteen years of experience with the worldwide accepted FIGO 2000, the present study provides a useful overview of its design and performance in a large nationwide cohort. Although the number of patients with resistance to single-agent therapy in the low-risk group made us inquisitive on possible improvements in the performance of FIGO 2000, exploration of possible improvement in classification is challenging when

only the prognostic factors currently employed in the FIGO 2000 are considered. Doppler ultrasonography, used to measure uterine vascularity through pulsatility index has been suggested as an independent prognostic factor for resistance to single-agent chemotherapy [24]. Further improvement by including novel variables such as Doppler pelvic ultrasonography should be considered. A renewed evaluation, preferably through international research collaboration would be needed to further validate these findings and refine FIGO 2000 into a straightforward classification system we could all embrace.

Conclusion

The total FIGO score is determined by a summation of scores for eight prognostic factors. The majority of factors relate to tumor bulk and are not independently prognostic for single-agent resistance. Our simplified alternative using only five of the FIGO prognostic factors remains an accurate system for discriminating patients requiring single as opposed to multi-agent chemotherapy. This simplified alternative would ideally reduce variability in scoring and improve agreement between centers. However, further validation is required to ascertain how this system performs in distinguishing ultra-high risk and high-risk patients.

Supplementary Table 1. FIGO 2000 Classification system GTN

Score	0	1	2	4
Age (years)	<40	≥40	-	-
Antecedent pregnancy	Mole	Abortion	Term	-
Interval (months)	<4	4-6	7-12	≥13
Pre-treatment serum hCG (IU/l)	$<10^3$	$< 10^4$	$< 10^5$	$> 10^5$
Tumor size (cm)	<3	3-4	≥5	-
Site of metastases	Lung	Spleen, kidney	Gastro-intestinal	Liver, brain
Number of metastases	-	1-4	5-8	>8
Previous failed chemotherapy	-	-	Single drug	Multi drug

A total score of 0-6 = low-risk; score ≥ 7 = high-risk

Supplementary Table 2. Characteristics of cases with discordant classification when simplified alternative were applied[a]

Case	Age	Antecedent	Interval	Pre-treatment serum hCG	Tumor size	Site of metastases	Number of metastases	Previous failed chemotherapy	Treatment	Conclusion
1	<40	Term	<4	1000-10,000	<3cm	Lung	1-4	None	MTX-ActD	False Positive
2	<40	Term	<4	1000-10,000	<3cm	Lung	1-4	None	EMA/CO	Unknown /similar
3	<40	Term	4-6	<1000	<3cm	Lung	1-4	None	MTX-EMA/CO	True Positive
4	<40	Mole	<4	>100,000	3-5cm	Lung	1-4	None	EMA/CO	Unknown /similar
5	<40	Abortion	>12	1000-10,000	3-5cm	None	0	Single	MTX-ActD	True Negative
6	<40	Term	>12	<1000	<3cm	Lung	1-4	None	EMA/CO-PAC/PLAT	False Negative
7	<40	Term	>12	1000-10,000	<3cm	None	0	None	MTX-ActD	Unknown
8	<40	Term	>12	1000-10,000	<3cm	None	0	None	EMA/CO	Unknown

[a] Numbers correspond to the case-numbers mentioned in Table 3, reflecting discordance between FIGO 2000 and simplified alternatives

Abbreviations: MTX: methotrexate; ActD: actinomycine D; EMA/CO: etoposide, methotrexate, actinomycine D alternating weekly with cyclophosphamide and vincristin; PAC/PLAT: paclitaxel, cisplatin

REFERENCES

1. Lurain JR. Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. *Am J Obstet Gynecol* 2010; **203**(6): 531-9.
2. Soper JT, Evans AC, Conaway MR, Clarke-Pearson DL, Berchuck A, Hammond CB. Evaluation of prognostic factors and staging in gestational trophoblastic tumor. *Obstet Gynecol* 1994; **84**(6): 969-73.
3. Berkowitz RS, Goldstein DP. Current management of gestational trophoblastic diseases. *Gynecol Oncol* 2009; **112**(3): 654-62.
4. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet* 2010; **376**(9742): 717-29.
5. Bagshawe KD. Risk and prognostic factors in trophoblastic neoplasia. *Cancer* 1976; **38**(3): 1373-85.
6. Azab MB, Pejovic MH, Theodore C, et al. Prognostic factors in gestational trophoblastic tumors. A multivariate analysis. *Cancer* 1988; **62**(3): 585-92.
7. Hancock BW. Staging and classification of gestational trophoblastic disease. *Best Pract Res Clin Obstet Gynaecol* 2003; **17**(6): 869-83.
8. Hancock BW, Welch EM, Gillespie AM, Newlands ES. A retrospective comparison of current and proposed staging and scoring systems for persistent gestational trophoblastic disease. *Int J Gynecol Cancer* 2000; **10**(4): 318-22.
9. Committee FO. FIGO staging for gestational trophoblastic neoplasia 2000. FIGO Oncology Committee. *Int J Gynaecol Obstet* 2002; **77**(3): 285-7.
10. Ngan HY, Bender H, Benedet JL, et al. Gestational trophoblastic neoplasia, FIGO 2000 staging and classification. *Int J Gynaecol Obstet* 2003; **83 Suppl 1**: 175-7.
11. Nevin J, Silcocks P, Hancock B, Coleman R, Nakielny R, Lorigan P. Guidelines for the stratification of patients recruited to trials of therapy for low-risk gestational trophoblastic tumor. *Gynecol Oncol* 2000; **78**(2): 92-6.
12. McGrath S, Short D, Harvey R, Schmid P, Savage PM, Seckl MJ. The management and outcome of women with post-hydatidiform mole 'low-risk' gestational trophoblastic neoplasia, but hCG levels in excess of 100 000 IU l(-1). *Br J Cancer* 2010; **102**(5): 810-4.
13. Seckl MJ, Sebire NJ, Fisher RA, et al. Gestational trophoblastic disease: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24 Suppl 6**: vi39-50.
14. Alifrangis C, Agarwal R, Short D, et al. EMA/CO for high-risk gestational trophoblastic neoplasia: good outcomes with induction low-dose etoposide-cisplatin and genetic analysis. *J Clin Oncol* 2013; **31**(2): 280-6.
15. Savage P, Kelpanides I, Tuthill M, Short D, Seckl MJ. Brain metastases in gestational trophoblast neoplasia: An update on incidence, management and outcome. *Gynecol Oncol* 2015; **137**(1): 73-6.
16. Eysbouts YK, Massuger L, Thomas C, et al. Dutch Risk Classification and FIGO 2000 for Gestational Trophoblastic Neoplasia Compared. *Int J Gynecol Cancer* 2016.
17. Ngan HY. The practicability of FIGO 2000 staging for gestational trophoblastic neoplasia. *Int J Gynecol Cancer* 2004; **14**(2): 202-5.
18. Cole LA, Kohorn EI, Kim GS. Detecting and monitoring trophoblastic disease. New perspectives on measuring human chorionic gonadotropin levels. *J Reprod Med* 1994; **39**(3): 193-200.

19. Chapman-Davis E, Hoekstra AV, Rademaker AW, Schink JC, Lurain JR. Treatment of non-metastatic and metastatic low-risk gestational trophoblastic neoplasia: factors associated with resistance to single-agent methotrexate chemotherapy. *Gynecol Oncol* 2012; **125**(3): 572-5.
20. Powles T, Young A, Sanitt A, et al. The significance of the time interval between antecedent pregnancy and diagnosis of high-risk gestational trophoblastic tumours. *Br J Cancer* 2006; **95**(9): 1145-7.
21. Altieri A, Franceschi S, Ferlay J, Smith J, La Vecchia C. Epidemiology and aetiology of gestational trophoblastic diseases. *Lancet Oncol* 2003; **4**(11): 670-8.
22. Altman AD, Bentley B, Murray S, Bentley JR. Maternal age-related rates of gestational trophoblastic disease. *Gynecol Oncol* 2008; **112**(2 Pt 1): 244-50.
23. Sebire NJ, Foskett M, Fisher RA, Rees H, Seckl M, Newlands E. Risk of partial and complete hydatidiform molar pregnancy in relation to maternal age. *BJOG* 2002; **109**(1): 99-102.
24. Agarwal R, Harding V, Short D, et al. Uterine artery pulsatility index: a predictor of methotrexate resistance in gestational trophoblastic neoplasia. *Br J Cancer* 2012; **106**(6): 1089-94.

Chapter 8

General discussion

Main aim of the present thesis was to further improve management of patients with GTN, through prediction of those patients requiring chemotherapeutic treatment and optimizing risk classification. With the introduction of effective chemotherapeutic treatment, GTN has become a highly curable disease with overall cure rates exceeding 90% [1-3]. To achieve this success, early detection and adequate treatment of GTN patients is essential, accompanied by accurate identification of those patients at risk of resistance to single-agent chemotherapy, since prompt treatment with more intensive combination chemotherapy improves survival and shortens treatment duration. Combination chemotherapy with EMA/CO as is recommended in case of high-risk disease, however is toxic, causing alopecia, myelosuppression and neuropathy and should therefore be applied with caution [4].

FOLLOW-UP AND DIAGNOSIS OF POST-MOLAR GTN

For women diagnosed with a molar pregnancy the post-molar GTN risk and subsequent need for further chemotherapeutic management is approximately 0.5-1% for partial hydatidiform moles and 15-20% for complete hydatidiform moles [4-8]. To enable early detection of patients with post-molar GTN, all patients with hydatidiform mole are subjected to recurrent hCG surveillance after molar evacuation [4]. Serum hCG is a reliable marker for the detection of post-molar GTN, which is expressed by a plateaued or increased serum hCG concentration [9,10]. **Chapter 3** presents a regression corridor for uneventful both complete and partial hydatidiform moles, updating the normogram as presented by Yedema et al. in 1993 [11]. This new hCG normogram is applicable as a reference in the first-trimester ultrasound era and provides guidance during follow-up of patients with both complete and partial hydatidiform moles after evacuation. Since the configuration of a normogram is as dynamic as the patient group it affects, and the type of assay used, changes in future practice may require new adjustments over time and use of the normogram should be restricted to samples measured at the laboratory of the Dutch Central Registry for Hydatidiform moles.

PREDICTION OF POST-MOLAR GTN

It is unknown why after molar evacuation, some hydatidiform moles develop into post-molar GTN, while others go into spontaneous regression. Clinical characteristics, such as advanced maternal age, large ovarian cysts and history of a prior mole have shown to be only weak predictors of post-molar GTN [1,12,13]. Most studies have therefore focused on the tumor marker hCG. Although a normogram can support the detection of post-molar GTN, the tool is not suitable for early identification of patients at high-risk of post-molar GTN. To identify a means to predict post-molar GTN, some investigators have tried to predict post-molar GTN through differences in hCG regression patterns [11,14-17], others evaluated possible differences in hCG ratios obtained at different time intervals [18,19] or serum hCG levels taken at a given time point after evacuation [18,20]. Although these studies emphasize the relevance of serum hCG as a reliable predictor of post-molar GTN, none of these findings were sufficiently accurate to guide individual management [11,15,16,18,19]. In **chapter 4** we present nomograms based on a single serum hCG value between 1 and 4 weeks after evacuation, to estimate the individual risk of post-molar GTN. All nomograms had good to excellent ability to distinguish between patients who will develop post-molar GTN and patients with uneventful hCG regression, reflected by a *c*-index value increasing from 0.83 in week 1, to 0.95 in week 4. The development of these nomograms resulted in a simple and reliable tool to estimate the individualized risk of post-molar GTN, based on a single serum hCG measurement. With the use of this tool, clinicians can provide proper counseling of all complete hydatidiform mole patients and identify patients at high-risk of post-molar GTN. Validation in an independent and preferably larger dataset is needed to further strengthen our findings. Trophoblastic centers worldwide however use different hCG assays with varying specificity for particular subtypes of hCG. An external validation of the prediction tool as presented in **chapter 4**, on a different patient group, but measured with the same hCG assay is therefore not feasible. An independent cohort with serum hCG values obtained using a different hCG assay can however be used to validate the approach of predicting post-molar GTN with the use of nomograms. In the United Kingdom, a non-commercial in-house hCG RIA assay, using a rabbit polyclonal antibodies is used for monitoring all GTD patients. With the use of this independent and considerably large dataset, one could validate whether the approach described in **chapter 4,** can be extended to a different assay.

UNIFORMITY IN CLASSIFICATION

The use of a clinical scoring system to classify GTN patients with recognized prognostic criteria was first described by Bashawe in 1976 [21]. He investigated 317 patients with GTN and identified ten factors associated with response to chemotherapy. Patients with GTN can subsequently be classified with low-risk or high-risk disease and treated accordingly. The derived weighted prognostic scoring system, formed the basis for many other classification systems such as the World Health Organization (WHO) classification system and more recently the FIGO 2000 [22-24]. Unlike the FIGO 2000 system, the Dutch classification system uses a small subset of absolute criteria to stratify patients into low or high-risk for developing single-agent resistance, including antecedent term pregnancy and previous failed chemotherapy [22,25]. Although this system provides relatively straightforward classification, it hampers the comparison of Dutch management results with those obtained worldwide [25]. **Chapter 5** described a retrospective comparison of the FIGO 2000 and the Dutch classification systems for patients with GTN. Both systems turned out to be widely equivalent with similar risk classification in up to 93.4% of cases, even though items and scoring of these items were quite different. Discordant risk distribution was predominantly seen in patients with antecedent term pregnancy. In the Netherlands term pregnancy in itself is considered an indicator for poor prognosis, whereas the FIGO 2000 system only allocates a relative weight to this criterion. Although a lower cure rate after term pregnancy has been reported in several studies, an intrinsic correlation between term pregnancy and other factors for poor prognosis such as the presence of widespread metastases, a prolonged interval between diagnosis and antecedent pregnancy and the involved tumor burden is likely present. The actual presence of these other prognostic factors in patients with antecedent term pregnancy may therefore be equally essential [26-30].

To realize comparison of therapy results with other nations, we propose replacement in the Netherlands of the Dutch classification system with the FIGO 2000 system. Considering the possibilities for simplification and improvement as suggested in **chapter 6**, replacement of the Dutch classification system should ideally involve a refined edition of the FIGO 2000 scoring system.

RISK CLASSIFICATION: IS LESS REALLY MORE?

The comparison of the Dutch classification system with the FIGO 2000 system in **chapter 5** has provided insight in the extensive overlap in classification of both systems. The relative abundance of criteria in the FIGO 2000 however made us inquisitive on the possibilities to simplify the system without compromising outcome. Many risk factors involved in the FIGO 2000 scoring system relate to tumor bulk and may not be independently prognostic [26,28,31,32]. **Chapter 6** presents a re-evaluation of all prognostic factors involved in the FIGO 2000 system and an examination whether simplification is feasible.

With the use of stepwise backwards (Wald) logistic regression a simplified risk scoring model was identified comprising the factors age, pre-treatment serum hCG, number of metastases, antecedent pregnancy and interval, thus omitting tumor size, previously failed chemotherapy and site of metastases. A reduction of the number of factors involved in the FIGO system will likely result in reduced variability in scoring and classification. Especially in a low incidence disease like GTN, unification between centers is essential to optimize management.

Using simplified model 3 as presented in **chapter 6**, identical classification in all but one patient was obtained when compared to FIGO 2000. Would elimination of the factors tumor size, previously failed chemotherapy and widespread metastases truly support agreement in classification when used in daily practice? Failure to respond to single-agent therapy always justifies the start of either a different single-agent regimen or a multi-agent regimen depending on serum hCG value. Confusion may however exist on the definition of previous failure of chemotherapy and scoring as such in the FIGO 2000 system. Would a single shot of MTX administered at an initial suspicion of extra-uterine gravidity imply previous failure of chemotherapy for GTN? In addition, although tumor size can be derived quite easily in a noninvasive manner, the volume of a trophoblastic tumor may not represent the proportion of viable cells in a tumor mass due to variations in the extent of necrosis and hemorrhage [33]. Trophoblastic tumor masses have a slow radiologic resolution, particularly with bigger masses. In several instances, opacities took more than a year to fully dissolve after the end of chemotherapy. In follow-up and restaging this should therefore be taken explicitly into consideration [21]. In uni- and multivariable analysis, we showed tumor

size to be a significant predictor of the need for salvage therapy in the low-risk group, which is in line with other studies [32]. In our study, all of these patients were fully salvaged. In addition, a tumor size of over 5 cm was also commonly seen in the single-agent responsive group. Proposing a prominent role for tumor size in the classification system by allotting additional points is therefore not the answer. Finally, considering the elimination of widespread metastases as a separate criterion in FIGO 2000, it is important to realize that the present FIGO score, whilst only designed for stratifying GTN patients to low and high-risk treatment, does give us an indication of the patient group at risk of early death after initiating chemotherapy, or late death from multi-drug resistant disease [29,34,35]. Patients with widespread disease, commonly reflected by a very high FIGO score (>12), are at significant risk of pulmonary, intra-peritoneal or intracranial hemorrhage and may benefit from low-dose induction chemotherapy. Especially liver and brain involvement reduces the long-term survival substantially [36-38]. Removal of site of metastases as a risk criterion would hinder identification of this patient group [39]. Consequently, a new system will need to be carefully evaluated with sufficient patient numbers in the high- and ultra-high-risk groups.

Altogether, the elimination of tumor size and previously failed chemotherapy would simplify the current classification system and could subsequently reduce variability in scoring and improve agreement. Considering the number of patients with resistance to single-agent chemotherapy in the low-risk group, the possibility to refine the FIGO classification system should additionally be evaluated. Although improvement appears challenging when only prognostic factors currently applied in the FIGO 2000 are considered, variables such as Doppler ultrasonography, used to measure uterine vascularity through pulsatility index have been suggested as an independent prognostic factor for resistance to single-agent chemotherapy [40]. If introduction of Doppler ultrasonography with simultaneous simplification of the model would result in a decline of non-responders in the low-risk group without compromising single-agent responders by erroneously assigning them to high-risk therapy in an independent dataset, a simple but refined classification could result.

RECOMMENDATIONS IN THE MANAGEMENT OF GTN

Consensus in terminology and diagnostic criteria

To acquire consensus in the management of GTN, standardizing risk classification is ideally accompanied by standardized terminology and diagnosis. GTN differs from most other solid tumors, for which histological confirmation is recommended [10]. The condition has subsequently received many denominators based on pathology, laboratory results and clinical behavior (e.g. gestational trophoblastic tumor, persistent trophoblastic disease, malignant GTD) [33]. To avoid confusion, it is recommended to use one general term for all patients with abnormal gestational trophoblastic proliferation requiring treatment for a potential or proven malignancy. At the FIGO 2000 meeting in 2003, the term gestational trophoblastic neoplasia (GTN) was therefore recommended to replace all other terminology [41]. Though this entity gradually reaches universal acceptance, differences in the diagnostic criteria for GTN still exist. The International Federation of Obstetrics and Gynecology (FIGO) defines GTN as: 1) plateau in hCG concentration in 4 consecutive blood samples over a period of 3 weeks and/or, 2) a rise in hCG concentration for 3 consecutive samples over a period of 2 weeks [42]. Some will include persistence of detectable hCG concentrations 6 months after evacuation as an additional criterion, while others include the pathological confirmation of choriocarcinoma as an absolute criterion [4,33,43]. Furthermore the actual cut-off values of hCG rise or plateau is still left to the discretion of the individual physician. Only by unifying the definition of diagnosis, meaningful comparison of management outcome is feasible. The following unequivocal criteria for GTN are therefore proposed, predominantly based on recommendations of the FIGO 2000 meeting: 1) a plateau in hCG concentration for 4 consecutive blood samples over a period of 3 weeks (defined as equivalent values not exceeding an overall 10% rise or decline over this 3 week period), 2) two consecutive rises in hCG concentration of 10% or more for at least 2 weeks, and 3) histopathological diagnosis of choriocarcinoma, as in this case the disease is unlikely to spontaneously disappear [6]. An elevated but falling hCG 6 months after evacuation should no longer be used as an absolute criterion, because with continuous surveillance, spontaneous serum hCG normalization eventually occurred in all of these patients [44,45].

Consensus in the use of investigative tools

When the diagnosis of GTN is established, usually through hCG follow-up, a workup with diagnosis of metastases and an evaluation for the presence of risk factors should be performed [43,46]. Although it is tempting to use more sophisticated technology such as computed tomography (CT) scans or magnetic resonance imaging (MRI) providing much better resolution than conventional imaging, chest X-ray is appropriate for the detection of lung metastases, since the presence of micro metastases does not influence management and outcome [47,48]. Only when lesions are detected on a chest X-ray, CT-thorax/abdomen and MRI of the brain are indicated to evaluate the presence of widespread disease, which significantly alters management and prognosis [6,44].

Regarding follow-up, a reliable serum hCG assay is essential for the management of patients with GTD. hCG is a heterogeneous molecule, produced by trophoblastic tissue during pregnancy [49]. hCG comprises an α-subunit (shared with other glycoprotein hormones including luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone) and a β-subunit that confers specificity [50]. Besides intact hCG, an assay used to monitor hCG values in patients with gestational trophoblastic disease should therefore target the β-subunit [51]. In the first trimester of healthy pregnancies, hCG is predominantly present in the native form. In trophoblastic disease, hCG can exist in a number of fragments. There are currently many commercial sandwich-type assays available for normal pregnancy. Commercial assays variously detect all forms of hCG, resulting in different results [52,53]. In the Netherlands, an in-house developed radioimmunoassay (RIA) designed to specifically detect both intact hCG and free β-subunit is used for all measurements in sera sent to the Dutch Central Registry for Hydatidiform Moles since 1977 [11]. Based on measurements performed with this specific immunoassay, several tools have been developed to guide follow-up and management of GTD patients, including the hCG regression curve discussed in **chapter 3** of this thesis and the prediction tool presented in **chapter 4** of this thesis. Since specificity for the different forms of hCG differs between hCG assays, results from these studies will only apply to measurements performed at laboratory of the Dutch Central Registry. Although the registry provides nationwide hCG measurement service for gynecologists, standard use of this assay is still not reached. Centralization of care in the Netherlands may induce standard use of this specific assay and thus enable uniformity in hCG measurement and subsequent management nationwide.

International registration and collaboration

The Dutch Central Registry for Hydatidiform Moles was established in 1977 and resides at the Radboud University Medical Center in the Netherlands. This voluntary registry mainly serves as an epidemiological database for all patients with gestational trophoblastic disease. Patients with GTN are quarterly discussed in the Dutch Working party on Trophoblastic Disease. In the United Kingdom, clinicians are obliged to register all patients diagnosed with trophoblastic disease centrally for hCG monitoring and follow-up since 1973. Only two hospitals in the UK are designated to treat patients with GTD. Today, the electronic database holds records of over 35,000 women with GTD and provides excellent data for research and audit [54,55]. Following these examples, Belgium, France and other European countries have set up a national registration system to guide physicians and improve the management of patients with GTD [56,57].

After wide agreement that a European network for clinicians and researchers working in the field of GTD would lead to an improvement in knowledge and management of patients with GTD, the European Organization for Treatment of Trophoblastic Diseases (EOTTD) was established in 2009. Facilitated by this organization, international guidelines have been formulated [6], centralized management of patients was encouraged and collaboration in research was stimulated [58]. Institution of an online database for GTD patients registered in all participating countries of the EOTTD would however truly take international research collaboration to a higher level. Especially in case of GTN, international multi-center collaborations are needed to study sufficient numbers of patients.

FUTURE PERSPECTIVE

The present thesis discusses simplification of the FIGO 2000 system through elimination of tumor size and previously failed chemotherapy as prognostic criteria (**chapter 6**). Ideally this should be accompanied by simultaneous improvement of the FIGO 2000 system, as a reduction of misclassified low-risk patients would result in reduced treatment duration and earlier permission for a renewed pregnancy. Doppler ultrasonography has been suggested as an independent predictor of MTX resistance in patients with a FIGO score of 5-6 [59]. To explore the prognostic value of Doppler ultrasonography for all low-risk GTN patients, this factor should be added to the

multivariable analyses in our dataset. If a reduced misclassification-rate could indeed be realized by adding this factor, the next step would be to consider all independent prognostic criteria involved in the FIGO 2000 and Doppler ultrasonography prospectively. **Chapter 5** discussed the milder characteristics of a number of patients with a FIGO 2000 score of 7, predominantly with tumor size exceeding 5 cm, pretreatment serum hCG exceeding 100,000 IU/l and age of over 40 years, but absence of serious prognostic factors such as widespread metastases. It is conceivable that this patient group presents a gray zone and some of these patients may have been responsive to single-agent chemotherapy alone. An evaluation of their response to single-agent chemotherapy may be considered to see if the more aggressive multi-agent regimen with significant toxicity could be avoided. Through international research collaboration, the possible response to single-agent chemotherapy can be evaluated for all patients with a FIGO score of 0-8 and absence of widespread metastatic disease. Single-agent chemotherapy as first-line therapy appears to be safe in this particular patient group. In Denmark, methotrexate is considered first-line treatment in all patients with non-PSTT/ETT GTN, with ActD or multi-agent chemotherapy as second- or third-line treatment of choice [60]. Patients with widespread disease and/or ultra-high risk disease should be commenced on multi-agent chemotherapy to ensure optimal chances of survival. In this new cohort of patients, a new multivariable logistic regression can subsequently be conducted, including all prognostic criteria involved in the simplified scoring system and Doppler ultrasonography measurements to identify independent risk factors for single-agent chemotherapy resistance. Besides a validation of the study results as presented in **chapter 6**, this will provide additional information regarding the possible response to single-agent chemotherapy in patients with a FIGO score of 7 or 8. All factors with independent prognostic value can be implemented in a renewed and further simplified system of factors, while eventual weighting of all individual factors can be derived from the regression coefficients in multivariable logistic regression analysis. Alongside further consensus and unification worldwide, this renewed classification system would ideally result in a reduced misclassification-rate.

Some additional steps should be made before implementation of such a system is completed. First of all, one should consider the threshold for low versus high-risk disease, ensuring adequate recognition of non-responders without considerable compromise on single-agent responders by erroneously assigning them to high-risk therapy. The Receiver Operating Characteristic (ROC) curve plotting the sensitivity

against the 1-specificity of this renewed system, will be helpful in decision of the optimal cutoff score for low versus high-risk disease. And finally, we will need to re-evaluate the threshold for ultra-high-risk patients as these patients may benefit from low-dose induction chemotherapy.

REFERENCES

1. Lurain JR. Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. *Am J Obstet Gynecol* 2010; **203**(6): 531-9.
2. Ngan HY, Kohorn EI, Cole LA, et al. Trophoblastic disease. *Int J Gynaecol Obstet* 2012; **119 Suppl 2**: S130-6.
3. Froeling FE, Seckl MJ. Gestational trophoblastic tumours: an update for 2014. *Curr Oncol Rep* 2014; **16**(11): 408.
4. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet* 2010; **376**(9742): 717-29.
5. Berkowitz RS, Goldstein DP. Clinical practice. Molar pregnancy. *N Engl J Med* 2009; **360**(16): 1639-45.
6. Seckl MJ, Sebire NJ, Fisher RA, et al. Gestational trophoblastic disease: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24 Suppl 6**: vi39-50.
7. Feltmate CM, Batorfi J, Fulop V, Goldstein DP, Doszpod J, Berkowitz RS. Human chorionic gonadotropin follow-up in patients with molar pregnancy: a time for reevaluation. *Obstet Gynecol* 2003; **101**(4): 732-6.
8. Hancock BW, Nazir K, Everard JE. Persistent gestational trophoblastic neoplasia after partial hydatidiform mole incidence and outcome. *J Reprod Med* 2006; **51**(10): 764-6.
9. Committee FO. FIGO staging for gestational trophoblastic neoplasia 2000. FIGO Oncology Committee. *Int J Gynaecol Obstet* 2002; **77**(3): 285-7.
10. Ngan HY, Bender H, Benedet JL, et al. Gestational trophoblastic neoplasia, FIGO 2000 staging and classification. *Int J Gynaecol Obstet* 2003; **83 Suppl 1**: 175-7.
11. Yedema KA, Verheijen RH, Kenemans P, et al. Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. *Am J Obstet Gynecol* 1993; **168**(3 Pt 1): 787-92.
12. Berkowitz RS, Bernstein MR, Laborde O, Goldstein DP. Subsequent pregnancy experience in patients with gestational trophoblastic disease. New England Trophoblastic Disease Center, 1965-1992. *J Reprod Med* 1994; **39**(3): 228-32.
13. Ayhan A, Tuncer ZS, Halilzade H, Kucukali T. Predictors of persistent disease in women with complete hydatidiform mole. *J Reprod Med* 1996; **41**(8): 591-4.
14. Schlaerth JB, Morrow CP, Kletzky OA, Nalick RH, D'Ablaing GA. Prognostic characteristics of serum human chorionic gonadotropin titer regression following molar pregnancy. *Obstet Gynecol* 1981; **58**(4): 478-82.
15. Shigematsu T, Kamura T, Saito T, Kaku T, Nakano H, Kinugawa N. Identification of persistent trophoblastic diseases based on a human chorionic gonadotropin regression curve by means of a stepwise piecewise linear regression analysis after the evacuation of uneventful moles. *Gynecol Oncol* 1998; **71**(3): 376-80.
16. Kim BW, Cho H, Kim H, et al. Human chorionic gonadotrophin regression rate as a predictive factor of postmolar gestational trophoblastic neoplasm in high-risk hydatidiform mole: a case-control study. *Eur J Obstet Gynecol Reprod Biol* 2012; **160**(1): 100-5.
17. Lybol C, Sweep FC, Ottevanger PB, Massuger LF, Thomas CM. Linear regression of postevacuation serum human chorionic gonadotropin concentrations predicts postmolar gestational trophoblastic neoplasia. *Int J Gynecol Cancer* 2013; **23**(6): 1150-6.

18. Kang WD, Choi HS, Kim SM. Prediction of persistent gestational trophobalstic neoplasia: the role of hCG level and ratio in 2 weeks after evacuation of complete mole. *Gynecol Oncol* 2012; **124**(2): 250-3.
19. van Trommel NE, Ngo Duc H, Massuger LF, et al. Early identification of persistent trophoblastic disease with serum hCG concentration ratios. *Int J Gynecol Cancer* 2008; **18**(2): 318-23.
20. Wolfberg AJ, Berkowitz RS, Goldstein DP, Feltmate C, Lieberman E. Postevacuation hCG levels and risk of gestational trophoblastic neoplasia in women with complete molar pregnancy. *Obstet Gynecol* 2005; **106**(3): 548-52.
21. Bagshawe KD. Risk and prognostic factors in trophoblastic neoplasia. *Cancer* 1976; **38**(3): 1373-85.
22. Hancock BW. Staging and classification of gestational trophoblastic disease. *Best Pract Res Clin Obstet Gynaecol* 2003; **17**(6): 869-83.
23. Hancock BW, Welch EM, Gillespie AM, Newlands ES. A retrospective comparison of current and proposed staging and scoring systems for persistent gestational trophoblastic disease. *Int J Gynecol Cancer* 2000; **10**(4): 318-22.
24. El-Helw LM, Coleman RE, Everard JE, et al. Impact of the revised FIGO/WHO system on the management of patients with gestational trophoblastic neoplasia. *Gynecol Oncol* 2009; **113**(3): 306-11.
25. Dijkema HE, Aalders JG, de Bruijn HW, Laurini RN, Willemse PH. Risk factors in gestational trophoblastic disease, and consequences for primary treatment. *Eur J Obstet Gynecol Reprod Biol* 1986; **22**(3): 145-52.
26. Soper JT, Evans AC, Conaway MR, Clarke-Pearson DL, Berchuck A, Hammond CB. Evaluation of prognostic factors and staging in gestational trophoblastic tumor. *Obstet Gynecol* 1994; **84**(6): 969-73.
27. Miller JM, Jr., Surwit EA, Hammond CB. Choriocarcinoma following term pregnancy. *Obstet Gynecol* 1979; **53**(2): 207-12.
28. Azab MB, Pejovic MH, Theodore C, et al. Prognostic factors in gestational trophoblastic tumors. A multivariate analysis. *Cancer* 1988; **62**(3): 585-92.
29. Lurain JR, Sciarra JJ. Study and treatment of gestational trophoblastic diseases at the John I. Brewer Trophoblastic Disease Center, 1962-1990. *Eur J Gynaecol Oncol* 1991; **12**(6): 425-8.
30. Lurain JR, Casanova LA, Miller DS, Rademaker AW. Prognostic factors in gestational trophoblastic tumors: a proposed new scoring system based on multivariate analysis. *Am J Obstet Gynecol* 1991; **164**(2): 611-6.
31. Kim SJ, Bae SN, Kim JH, et al. Effects of multiagent chemotherapy and independent risk factors in the treatment of high-risk GTT--25 years experiences of KRI-TRD. *Int J Gynaecol Obstet* 1998; **60 Suppl 1**: S85-96.
32. Nevin J, Silcocks P, Hancock B, Coleman R, Nakielny R, Lorigan P. Guidelines for the stratification of patients recruited to trials of therapy for low-risk gestational trophoblastic tumor. *Gynecol Oncol* 2000; **78**(2): 92-6.
33. Ngan HY. The practicability of FIGO 2000 staging for gestational trophoblastic neoplasia. *Int J Gynecol Cancer* 2004; **14**(2): 202-5.
34. Soper JT, Clarke-Pearson DL, Berchuck A, Rodriguez G, Hammond CB. 5-day methotrexate for women with metastatic gestational trophoblastic disease. *Gynecol Oncol* 1994; **54**(1): 76-9.

35. Bower M, Newlands ES, Holden L, et al. EMA/CO for high-risk gestational trophoblastic tumors: results from a cohort of 272 patients. *J Clin Oncol* 1997; **15**(7): 2636-43.

36. Ahamed E, Short D, North B, Savage PM, Seckl MJ. Survival of women with gestational trophoblastic neoplasia and liver metastases: is it improving? *J Reprod Med* 2012; **57**(5-6): 262-9.

37. Neubauer NL, Latif N, Kalakota K, et al. Brain metastasis in gestational trophoblastic neoplasia: an update. *J Reprod Med* 2012; **57**(7-8): 288-92.

38. Newlands ES, Holden L, Seckl MJ, McNeish I, Strickland S, Rustin GJ. Management of brain metastases in patients with high-risk gestational trophoblastic tumors. *J Reprod Med* 2002; **47**(6): 465-71.

39. Alifrangis C, Agarwal R, Short D, et al. EMA/CO for high-risk gestational trophoblastic neoplasia: good outcomes with induction low-dose etoposide-cisplatin and genetic analysis. *J Clin Oncol* 2013; **31**(2): 280-6.

40. Agarwal R, Harding V, Short D, et al. Uterine artery pulsatility index: a predictor of methotrexate resistance in gestational trophoblastic neoplasia. *Br J Cancer* 2012; **106**(6): 1089-94.

41. Ngan HY, Odicino F, Maisonneuve P, et al. Gestational trophoblastic diseases. *Int J Gynaecol Obstet* 2003; **83 Suppl 1**: 167-74.

42. Benedet JL, Bender H, Jones H, 3rd, Ngan HY, Pecorelli S. FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. FIGO Committee on Gynecologic Oncology. *Int J Gynaecol Obstet* 2000; **70**(2): 209-62.

43. Ngan HY, Seckl MJ, Berkowitz RS, et al. Update on the diagnosis and management of gestational trophoblastic disease. *Int J Gynaecol Obstet* 2015; **131 Suppl 2**: S123-6.

44. Mangili G, Lorusso D, Brown J, et al. Trophoblastic disease review for diagnosis and management: a joint report from the International Society for the Study of Trophoblastic Disease, European Organisation for the Treatment of Trophoblastic Disease, and the Gynecologic Cancer InterGroup. *Int J Gynecol Cancer* 2014; **24**(9 Suppl 3): S109-16.

45. Golfier F. Persistently raised and falling hCG at 6 months no longer a criterion for post molar gestational trophoblastic neoplasia? *BJOG* 2016; **123**(7): 1182.

46. Berkowitz RS, Goldstein DP. Current management of gestational trophoblastic diseases. *Gynecol Oncol* 2009; **112**(3): 654-62.

47. Darby S, Jolley I, Pennington S, Hancock BW. Does chest CT matter in the staging of GTN? *Gynecol Oncol* 2009; **112**(1): 155-60.

48. Goldstein DP, Garner EI, Feltmate CM, Berkowitz RS. The role of repeat uterine evacuation in the management of persistent gestational trophoblastic disease. *Gynecol Oncol* 2004; **95**(3): 421-2.

49. Thomas CM, Reijnders FJ, Segers MF, Doesburg WH, Rolland R. Human choriogonadotropin (hCG): comparisons between determinations of intact hCG, free hCG beta-subunit, and "total" hCG + beta in serum during the first half of high-risk pregnancy. *Clin Chem* 1990; **36**(4): 651-5.

50. Cole LA, Kohorn EI. The need for an hCG assay that appropriately detects trophoblastic disease and other hCG-producing cancers. *J Reprod Med* 2006; **51**(10): 793-811.

51. van Trommel NE, Sweep FC, Schijf CP, Massuger LF, Thomas CM. Diagnosis of hydatidiform mole and persistent trophoblastic disease: diagnostic accuracy of total human chorionic gonadotropin (hCG), free hCG {alpha}- and {beta}-subunits, and their ratios. *Eur J Endocrinol* 2005; **153**(4): 565-75.

52. Cole LA. Phantom hCG and phantom choriocarcinoma. *Gynecol Oncol* 1998; **71**(2): 325-9.
53. Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women with false-positive human chorionic gonadotropin concentrations. *Lancet* 2000; **355**(9205): 712-5.
54. Sebire NJ, Foskett M, Short D, et al. Shortened duration of human chorionic gonadotrophin surveillance following complete or partial hydatidiform mole: evidence for revised protocol of a UK regional trophoblastic disease unit. *BJOG* 2007; **114**(6): 760-2.
55. Imperial College Healthcare NHS. https://www.imperial.nhs.uk/our-services/cancer-services/gestational-trophoblastic-disease.
56. Belgian Register for GTD. http://www.mole-chorio-bgog.eu/nl/.
57. Web based resource for clinical trials. https://clinicaltrials.gov/ct2/show/NCT02892877.
58. The European Organisation for Treatment of Trophoblastic Diseases.
59. Sita-Lumsden A, Medani H, Fisher R, et al. Uterine artery pulsatility index improves prediction of methotrexate resistance in women with gestational trophoblastic neoplasia with FIGO score 5-6. *BJOG* 2013; **120**(8): 1012-5.
60. Niemann I, Vejerslev LO, Froding L, et al. Gestational trophoblastic diseases - clinical guidelines for diagnosis, treatment, follow-up, and counselling. *Dan Med J* 2015; **62**(11): A5082.

Chapter 9

Summary - Samenvatting

SUMMARY

Gestational trophoblastic disease (GTD) comprises a group of uncommon conditions associated with abnormal pregnancy. Histologically it includes the premalignant conditions of complete and partial hydatidiform mole, as well as the malignant counterparts choriocarcinoma, placental site trophoblastic tumor and epithelioid trophoblastic tumor. The malignant counterparts are collectively referred to as gestational trophoblastic neoplasia (GTN).

Chapter 1 reviews the most recent insights in epidemiology, diagnosis, treatment modalities and prognosis of patients with GTN, serving as an introduction to the studies performed and described in this thesis.

In **chapter 2** the trends in incidence of gestational trophoblastic disease in the Netherlands are determined using population-based data from PALGA, a national pathology database containing all histopathological records in the Netherlands. In the 20-year period between 1994 and 2013, 6341 cases of GTD were registered, representing an overall incidence rate of 1.66 per 1000 deliveries per year. An initial rise in incidence rate over the first ten years (increase per year 0.075, 95% CI 0.040-0.109 per 1000 deliveries per year) was followed by a stabilization from 2004 to 2013 (increase per year 0.011, 95% CI -0.017-0.040 per 1000 deliveries per year). From 2009 onwards a comparable incidence was reached for both complete and partial hydatidiform moles (incidence rates of 0.68 and 0.64 per 1000 deliveries respectively) and unspecified hydatidiform mole diagnosis declined significantly (from 0.14 per 1000 deliveries in 2003 to 0.03 per 1000 deliveries in 2013). The introduction of ancillary techniques such as $p57^{kip2}$ and ploidy analysis have likely improved the accuracy of diagnosis and therefore the actual estimates of GTD cases as registered by the Dutch pathological database (PALGA). As pathological confirmation is currently the norm and advanced pathological techniques are now widely available, reliable steady incidence rates may now have been reached.

The glycoprotein hormone human chorionic gonadotropin (hCG), produced by trophoblastic tissue, is a sensitive marker for monitoring trophoblastic activity in pregnancy and gestational trophoblastic disease. hCG surveillance after evacuation of a hydatidiform mole is essential in the management of GTD. In **chapter 3** a new serum

hCG normogram is proposed, based on 639 patients with both uneventful complete or partial hydatidiform moles between 1990 and 2014. So far, Dutch clinicians relied on a normogram as presented by Yedema in 1993, based on a historical cohort of patients with uneventful regression after a complete hydatidiform moles alone. Compared to the historical cohort of Yedema et al., lower pre-evacuation and follow-up serum hCG concentrations were observed in our study cohort. Moreover, a small but significant divergence in hCG regression between complete and partial hydatidiform moles was seen (median hCG normalization time of seven weeks and six weeks respectively, $p<0.001$). As actual differences in regression only encompassed days, we proposed the use of a combined normogram for both complete and partial hydatidiform moles to promote clarity and unity in daily practice. The normogram replaces the regression corridor as presented by Yedema et al. and is applicable as a reference guideline for follow-up after evacuation of a hydatidiform mole in the first-trimester ultrasound era.

Chapter 4 presents a nomogram for patients with hydatidiform moles, to enable early identification of patients at high-risk of post-molar GTN. Between 1990 and 2014 serum hCG values, taken 1 to 4 weeks after evacuation were retrospectively evaluated for patients with uneventful serum hCG regression and patients with post-molar GTN. Logistic regression was used to generate nomograms for every week after evacuation, based on serum hCG measurements at that given week. The discriminative ability of the prognostic models, or the ability to distinguish women with low-risk of post-molar GTN from women with high-risk of post-molar GTN, was expressed by means of the concordance index (*c*-index). For a binary outcome, *c* is identical to the area under the receiver-operating characteristics (ROC) curve (AUC). With a *c*-index value ranging from 0.83 in week 1, to 0.95 in week 4, all nomograms had good to excellent ability to distinguish women with low-risk of post-molar GTN from women with high-risk of post-molar GTN. The development of these nomograms resulted in a simple and reliable tool to estimate the individualized risk of post-molar GTN, based on a single serum hCG measurement. Early start of chemotherapeutic treatment may be beneficial in patients at high-risk of post-molar GTN, especially in case of poor compliance to follow-up.

The possible benefits of hysterectomy on treatment duration and treatment toxicity for patients with GTN are explored in **Chapter 5**. Although chemotherapy is gener-

ally the treatment of choice in patients with GTN, hysterectomy is still considered in selected cases. All patients diagnosed with GTN and treated with a hysterectomy between 1977 and 2012, were identified from the Dutch Central Registry for Hydatidiform Moles and records of the Dutch Working party of Trophoblastic Disease. One hundred and nine patients (16.5% of all registered patients with GTN) underwent hysterectomy as part of their management for GTN. The majority of these patients was classified as low-risk disease (74.3%), post-molar GTN (73.5%) and disease confined to the uterus (65.1%). After hysterectomy alone, complete remission was achieved in 66.2% of patients with localized disease and in 15.8% of patients with metastatic disease. For patients with localized disease, treated with primary hysterectomy, treatment duration was significantly shorter (mean 3.2 weeks and 8.0 weeks respectively, $p=0.01$) with lower number of administered chemotherapy courses (mean 1.5 and 5.8 respectively, $p<0.01$) than patients in a matched control group. We therefore concluded that hysterectomy can still be considered an effective treatment option to either reduce or eliminate tumor bulk in selected cases. Primary hysterectomy should be considered mainly for patients over forty years of age with localized disease and no desire to preserve fertility, whereas patients with chemotherapy-resistant disease may benefit from additional hysterectomy, especially when disease is localized. For patients with widespread metastatic disease, the benefit of hysterectomy generally lies in the removal of chemotherapy-resistant tumor bulk to improve survival.

Once the diagnosis of GTN has been made, different prognostic classification systems can be used to stratify patients with GTN to single- of multi-agent chemotherapy. To validate and improve management of GTN between nations, it is essential to reach consensus on the used classification system and subsequent management protocols. **Chapter 6** describes a comparison between the Dutch classification system and the internationally used FIGO 2000 for patients with GTN. All patients diagnosed with GTN between 2003 and 2012 at the Trophoblastic Center in London and originally classified according to the FIGO 2000, were retrospectively scored according to the Dutch classification system. Risk classification with both systems was equivalent in 93.4% of cases, even though items and scoring were quite distinct. The Dutch system seems to be more simple and easier to apply, a degree of overtreatment can however be presumed with the use of either system. Considering the extensive overlap in risk factors involved in FIGO 2000, we concluded that further refinement of FIGO 2000

through a renewed univariate and multivariable analysis of the FIGO prognostic criteria would be useful.

In **chapter 7** we re-evaluate all prognostic criteria involved in the FIGO 2000 classification system and examine whether simplification of this system is feasible. A total of 813 patients diagnosed with GTN between 2003 and 2012 at the Trophoblastic Center in London and scored using FIGO 2000 were identified. Multivariable analysis and stepwise logistic regression were carried out to evaluate whether FIGO 2000 could be simplified. Of the eight FIGO risk factors only pre-treatment serum hCG levels exceeding 10,000 IU/l (OR = 5.0; CI 2.5-10.4) and 100,000 IU/l (OR = 14.3; CI 4.7-44.1), interval exceeding 7 months since antecedent pregnancy (OR = 4.1; CI 1.0-16.2) and tumor size of over 5 cm (OR = 2.2; CI 1.3-3.6) were identified as independent predictive factors for single-agent resistance. Stepwise logistic regression identified a simplified risk scoring model comprising age, pre-treatment serum hCG, number of metastases, antecedent pregnancy and interval, thus omitting tumor size, previous failed chemotherapy and site of metastases. With this model only 1 out of 725 patients was classified different from the FIGO 2000 system. Our simplified alternative using only five of the FIGO prognostic factors appears to be an accurate system for discriminating patients requiring single as opposed to multi-agent chemotherapy.

Chapter 8 summarizes and discusses the main findings of the present thesis. We especially stressed the need for consensus in terminology, diagnosis and treatment. Since multi-center and international collaboration are the only means to study sufficient number of patients in a low-incidence disease like GTN, variability in definition, diagnosis and treatment should be kept to an absolute minimum. To realize cross comparison of treatment results with other nations we propose replacement of the Dutch risk classification system with FIGO 2000. Considering the possibilities for simplification and improvement of FIGO, the replacement should ideally involve a refined edition. Considerations for further exploration of the refined FIGO with a proposal for future research is discussed.

SAMENVATTING

De term trofoblastziekten (gestational trophoblastic disease, GTD) omvat een groep zeldzame aandoeningen die geassocieerd is met een abnormale zwangerschap. Histologisch gezien, behoren hiertoe zowel de premaligne aandoeningen complete en partiële mola, als de maligne tegenhangers choriocarcinoom, placental site trophoblastic tumor en epithelioid trophoblastic tumor. Deze maligne aandoeningen worden gezamenlijk ook trofoblasttumor, of internationaal gestational trophoblastic neoplasia (GTN) genoemd.

Hoofdstuk 1 beschrijft de meest recente inzichten in epidemiologie, diagnose, behandeling en prognose van patiënten met GTN, als introductie op de studies die zijn uitgevoerd en beschreven in dit proefschrift.

In **hoofdstuk 2** worden de trends in incidentie van trofoblastziekten (GTD) in Nederland beschreven, met behulp van op populatie gebaseerde data die werd verkregen uit PALGA, een landelijke databank met alle pathologie-uitslagen in Nederland. In een periode van 20 jaar, tussen 1994 en 2013, werden 6341 gevallen van GTD geregistreerd, wat resulteert in een incidentie van 1.66 per 1000 bevallingen per jaar. Een initiële toename in incidentie in de eerste tien jaar (toename per jaar van 0.075, 95% CI 0.040-0.109 per 1000 bevallingen per jaar) werd daarbij gevolgd door een stabilisatie van 2004 tot 2013 (toename per jaar 0.011, 95% CI -0.017-0.040 per 1000 bevallingen per jaar). Vanaf 2009 waren de incidentie voor complete en partiële hydatidiforme mola hydatidosa vergelijkbaar (incidentie van respectievelijk 0.68 en 0.64 per 1000 bevallingen) en was sprake van een significante afname in ongespecificeerde mola hydatidosa (van 0.14 per 1000 bevallingen in 2003 naar 0.03 per 1000 bevallingen in 2013). De introductie van aanvullende technieken zoals $p57^{kip2}$ en ploidie-analyse hebben waarschijnlijk bijgedragen aan de toegenomen accuraatheid in diagnose en daarmee de daadwerkelijke bepaling van GTD gevallen zoals geregistreerd door de Nederlandse pathologie database (PALGA). Aangezien pathologische bevestiging van de diagnose momenteel de norm is en aanvullende pathologische technieken ruimschoots beschikbaar zijn, is hiermee mogelijk een betrouwbaar en stabiel incidentie cijfer bereikt.

Het glycoproteïne hormone human chorionic gonadotropin (hCG), geproduceerd door trofoblastweefsel, is een sensitieve merkstof om trofoblastactiviteit in zowel gezonde zwangerschappen als GTD te vervolgen. hCG controle na evacuatie van een mola hydatidosa is essentieel in de behandeling van GTD. In **hoofdstuk 3** wordt een nieuw serum hCG normogram voorgelegd, gebaseerd op 639 patiënten met spontane normalisatie van hCG concentraties na een complete of partiële mola hydatidosa tussen 1990 en 2014. Tot op heden waren Nederlandse clinici voor follow-up en diagnose afhankelijk van een normogram zoals gepresenteerd door Yedema in 1993. Dit normogram is gebaseerd op een historisch cohort van alleen complete mola hydatidosa patiënten met spontane normalisatie in hCG concentratie. In vergelijking tot het historische cohort van Yedema, zagen wij lagere pre-evacuatie en follow-up serum hCG concentraties in onze studie groep. Daarbij bleek sprake van een klein maar significant verschil in hCG regressie tussen complete en partiële mola hydatidosa (mediane hCG normalisatie tijd van respectievelijk zeven en zes weken, $p<0.001$). Aangezien de daadwerkelijke verschillen in regressie uiteindelijk slechts enkele dagen betreffen, adviseren wij het gebruik van een gecombineerd normogram voor zowel complete als partiële mola hydatidosa, ter bevordering van een heldere en uniforme werkwijze in de dagelijkse praktijk. Dit normogram zal de regressie corridor zoals gepresenteerd door Yedema vervangen en is ook in de huidige praktijk waar routine eerste-trimester echoscopie de norm is, toepasbaar als referentiekader voor follow-up van patiënten met mola hydatidosa na curettage.

Hoofdstuk 4 presenteert een nomogram om vroege identificatie van patiënten met een hoog risico op post-mola GTN mogelijk te maken. Hiertoe werd een retrospectieve analyse verricht van alle serum hCG waarden, afgenomen tussen 1 en 4 weken na evacuatie bij patiënten met spontane regressie en patiënten met post-mola GTN. Door middel van logistische regressie analyse werd vervolgens, gebaseerd op een enkele serum hCG meting in die desbetreffende week een nomogram gegenereerd. Het onderscheidend vermogen van de prognostische modellen, ofwel de mogelijkheid om vrouwen met laag-risico op post-mola GTN van vrouwen met hoog-risico op post-mola GTN te onderscheiden, werd uitgedrukt in de concordance index *(c-index).* Voor binaire uitkomsten, is c identiek aan de oppervlakte onder de receiver-operating characteristics (ROC) curve (AUC). Met een c-index waarde variërend van 0.83 in week 1, tot 0.95 in week 4, bleken alle nomogrammen een uitstekend vermogen te hebben om vrouwen met laag-risico op post-mola GTN van vrouwen met hoog-risico op post-

mola GTN te onderscheiden. De ontwikkeling van deze nomogrammen resulteerden dan ook in een eenvoudige, maar betrouwbare tool om het individuele risico op post-mola GTN, gebaseerd op een enkele hCG meting, in te schatten. Vroege start van chemotherapeutische behandeling kan van meerwaarde zijn bij patiënten met een hoog-risico op post-mola GTN, vooral als sprake is van matige compliance voor follow-up.

De mogelijke voordelen van een hysterecomie op de behandelduur en toxiciteit van behandeling voor patiënten met GTN worden besproken in **hoofdstuk 5.** Hoewel chemotherapie in het algemeen de eerste behandelkeuze is bij patiënten met GTN, kan hysterectomie nog steeds overwogen worden in specifieke gevallen. Alle patiënten die tussen 1977 en 2012 gediagnosticeerd werden met GTN en behandeld met hysterectomie, werden geselecteerd uit de Nederlandse Centrale Molaregistratie en het archief van de Nederlandse Tumorwerkgroep voor Trofoblastziekten. In totaal ondergingen 109 GTN patiënten (16.5% van alle geregistreerde patiënten met GTN) een hysterectomie als onderdeel van hun behandeling. Het merendeel van deze patiënten was geclassificeerd als laag-risico (74.3%), post-mola GTN (73.5%) met ziekte beperkt tot de uterus (65.1%). Na hysterectomie alleen werd in 66.2% van de patiënten met gelokaliseerde ziekte en in 15.8% van de patiënten met gemetastaseerde ziekte complete remissie bereikt. Bij patiënten met gelokaliseerde ziekte, behandeld met hysterectomie , was sprake van een significant kortere behandelduur (gemiddeld respectievelijk 3.2 weken en 8.0 weken, p=0.01), met minder benodigde chemokuren (gemiddeld respectievelijk 1.5 en 5.8 cycli, p<0.01) dan een gematchte controle Group. Wij concludeerden daarom dat hysterectomie in specifieke gevallen nog steeds beschouwd kan worden als een effectieve behandeloptie om tumor bulk te elimineren dan wel reduceren. Primaire hysterectomie kan overwogen worden bij patiënten ouder dan veertig jaar zonder kinderwens, met gelokaliseerde ziekte. Patiënten met chemo-resistente ziekte kunnen baat hebben bij aanvullende hysterectomie, vooral wanneer de ziekte zich beperkt tot de uterus. Voor patiënten met gemetastaseerde ziekte ligt het voordeel van een hysterectomie met name in het verwijderen van chemo-resistente tumorbulk ter bevordering van de overleving.

Wanneer de diagnose GTN gesteld is, kunnen verschillende prognostische classificatiesystemen gebruikt worden om patiënten met GTN te stratificeren voor mono- of polychemotherapie. Wanneer men behandelstrategieën tussen verschillende inter-

national centra wil vergelijken, is het echter essentieel om consensus te bereiken over het te gebruiken classificatiesysteem en het daarbij behorende behandelprotocol. **Hoofdstuk 6** beschrijft een vergelijking tussen het Nederlandse classificatie system en het international gebruikte FIGO 2000 classificatiesysteem voor patiënten met GTN. Alle patiënten, gediagnosticeerd tussen 2003 en 2012 in het Trophoblastic Center in London, aanvankelijk geclassificeerd volgens FIGO 2000, werden in retrospect geclassificeerd volgens het Nederlandse classificatiesysteem. Ondanks de verschillen in gebruikte items en methode van scoren, werd in 93.4% van de patiënten een overeenkomstige risicoclassificatie gezien. Hoewel het Nederlandse systeem eenvoudiger is in gebruik, wordt in beide systemen een mate van overbehandeling verondersteld. Gezien de grote mate van overlap tussen de risicofactoren van het FIGO 2000 classificatie systeem, concludeerden wij dat verdere verfijning van FIGO 2000, middels hernieuwde univariate en multivariable analyse van de prognostische FIGO criteria zinvol zou zijn.

In **hoofdstuk 7** werd een re-evaluatie van alle FIGO criteria verricht om de eventuele mogelijkheid tot vereenvoudiging van het FIGO 2000 classificatiesysteem te beoordelen. Achthonderddertien GTN patiënten, gediagnosticeerd tussen 2003 en 2012 in het Trophoblastic Center in London en gescored volgens FIGO 2000 werden geïdentificeerd. Met behulp van multivariable analyse en stepwise logistische regressie analyse werd vervolgens beoordeeld of vereenvoudiging van FIGO 2000 mogelijk was. Van de acht FIGO factoren werden alleen een serum hCG voor behandeling van meer dan 10,000 IU/l (OR = 5.0; CI 2.5-10.4) en 100,000 IU/l (OR = 14.3; CI 4.7-44.1), interval groter dan 7 maanden na antecedente zwangerschap, (OR = 4.1; CI 1.0-16.2) en een tumor groter dan 5 cm (OR = 2.2; CI 1.3-3.6) geïdentificeerd als onafhankelijke voorspellers voor het ontwikkelen van resistentie voor mono-chemotherapie. Met behulp van stepwise logistische regressie werd een vereenvoudigd classificatie systeem ontwikkeld, gebaseerd op leeftijd, serum hCG voor behandeling, aantal metastasen, antecedente zwangerschap en interval, maar zonder tumor grootte, eerder gefaalde chemotherapie en lokalisatie van metastasen. Met dit model werd slechts 1 van de in totaal 725 patiënten anders geclassificeerd dan volgens FIGO 2000. Hoewel het vereenvoudigde alternatief voor FIGO slechts gebruik maakt van vijf FIGO criteria, blijkt dit systeem op accurate wijze onderscheid te kunnen maken tussen patiënten die mono- dan wel polychemotherapy nodig hebben.

Hoofdstuk 8 vat de belangrijkste bevindingen van dit proefschrift samen en bediscussieerd deze bevindingen in bredere zin. Dit hoofdstuk richt zich daarbij vooral op het belang van consensus in terminologie, diagnose en behandeling. Juist bij een zeldzame aandoening als GTN, waarbij multi-center onderzoek en internationale samenwerking de enige mogelijkheid bieden om tot voldoende aantallen te komen, is het van groot belang om variaties in definitie, diagnose en ook behandelkeuzes zoveel mogelijk te beperken. Om vergelijking van Nederlandse behandelresultaten met andere landen mogelijk te maken, adviseren wij daarom vervanging van het Nederlandse risico classificatie system voor GTN door het FIGO 2000 classificatie system. Gezien de mogelijkheden tot vereenvoudiging en verbetering van dit FIGO systeem, zou deze vervanging bij voorkeur een verder verfijnde versie omvatten. Overwegingen om tot een verbeterde FIGO te komen worden tot slot besproken met suggesties voor toekomstig onderzoek.

Appendix

Bibliography
Dankwoord
Curriculum Vitae

BIBLIOGRAPHY

Papers

Eysbouts YK, Ottevanger PB, Massuger LF, IntHout J, Short D, Harvey R, Kaur B, Sebire NJ, Sarwar N, Sweep FC, Seckl MJ. Can the FIGO 2000 scoring system for gestational trophoblastic neoplasia (GTN) be simplified? A new retrospective analysis from a nationwide data-set. *Annals of Oncology* **2017** Ahead of print.

Eysbouts YK, Massuger LF, IntHout J, Lok CA, Sweep FC, Ottevanger PB. The added value of hysterectomy in the management of gestational trophoblastic neoplasia. *Gynecol Oncol* **2017** Ahead of print.

Eysbouts YK, Brouwer HB, Ottevanger PB, Massuger LF, Sweep FC, Thomas CM, van Herwaarden AE. Serum human chorionic gonadotropin (hCG) normogram for the detection of gestational trophoblastic neoplasia. *Int J Gynecol Cancer* **2017**; Epub ahead of print.

Eysbouts YK, Massuger LF, Thomas CM, Ottevanger PB, Short D, Harvey R, Sebire N, Kaur B, Naveed S, Sweep FC, Seckl MJ. Dutch risk classification and FIGO 2000 for gestational trophoblastic neoplasia compared. *Int J Gynecol Cancer* **2016** Nov; 26(9): 1712-1716.

Eysbouts YK, Bulten J, Ottevanger PB, Thomas CM, Ten Kate-Booij MJ, van Herwaarden AE, Siebers AG, Sweep FC, Massuger LF. Trends in incidence for gestational trophoblastic disease over the last 20 years in a population-based study. *Gynecol Oncol* **2016** Jan; 140(1):70-5.

Eysbouts YK, de Bie-Rocks B, van Dijk J, Nap AW. Characteristics, expectations and needs of the Dutch endometriosis society members. *Gynecol Obstet Invest* **2015**;79(4):234-8.

Eysbouts YK, Poulton A, Salmelainen P. Stimulant medication in pre-school children in New South Wales. *J. Paediatr Child Health* **2011** Dec;47(12): 870-4.

Eysbouts YK, Massuger LF, Ottevanger PB, IntHout J, Sweep FC, Thomas CM. Early prediction of post-molar gestational trophoblastic neoplasia with serum human chorionic gonadotropin. *In press*.

Abstracts

Eysbouts YK, Ottevanger PB, Massuger LF, IntHout J, Short D, Harvey R, Kaur B, Sebire NJ, Sarwar N, Sweep FC, Seckl MJ. Can the FIGO 2000 scoring system for gestational trophoblastic neoplasia (GTN) be simplified? A new retrospective analysis from a nationwide data-set. Oral presentation at the 19th Biannual World Congress of the International Society of the Study of Trophoblastic Diseases, Amsterdam, The Netherlands, September 22, 2017.

Eysbouts YK, Massuger LF, Ottevanger PB, IntHout J, Trommel NE, Sweep FC, Thomas CM. Early prediction of post-molar gestational trophoblastic neoplasia with the use of a single serum human chorionic gonadotropin measurement. Oral presentation at the 19th Biannual World Congress of the International Society of the Study of Trophoblastic Diseases, Amsterdam, The Netherlands, September 22, 2017.

Eysbouts YK, Massuger LF, Thomas CM, Ottevanger PB, Short D, Harvey R, Sebire N, Kaur B, Naveed S, Sweep FC, Seckl MJ. Dutch risk classification and FIGO 2000 for gestational trophoblastic neoplasia compared. Oral presentation at the 16th Biennial Meeting of the International Gynecological Cancer Society (IGCS), Lissabon, Portugal, October 31, 2016.

Eysbouts YK, Ottevanger PB, Massuger LF, IntHout J, Short D, Harvey R, Kaur B, Sebire NJ, Sarwar N, Sweep FC, Seckl MJ. Can the FIGO 2000 scoring system for gestational trophoblastic neoplasia (GTN) be simplified? A new retrospective analysis from a nationwide data-set. Oral presentation at the 16th Biennial Meeting of the International Gynecological Cancer Society (IGCS), Lissabon, Portugal, October 30, 2016.

Eysbouts YK, Bulten J, Ottevanger PB, Thomas CM, Ten Kate-Booij MJ, van Herwaarden AE, Siebers AG, Sweep FC, Massuger LF. Trends in incidence for gestational trophoblastic disease over the last 20 years in a population-based study. Oral presentation at

the 19th International Meeting of the European Society of Gynaecological Oncology (ESGO), Nice, Frankrijk, October 26, 2015.

Eysbouts YK, Massuger LF, Thomas CM, Ottevanger PB, Sweep FC. The clinical implications of hCG assays; case presentation. Oral presentation at the European Organisation for Treatment of Trophoblastic Diseases, Amsterdam, April 30, 2015.

Eysbouts YK, Massuger LF, IntHout J, Lok CA, Sweep FC, Ottevanger PB. The added value of hysterectomy in the management of gestational trophoblastic neoplasia. Poster presentation at the 19th Biannual World Congress of the International Society of the Study of Trophoblastic Diseases, Amsterdam, The Netherlands, September 24, 2017.

Eysbouts YK, Massuger LF, Thomas CM, Ottevanger PB, Short D, Harvey R, Sebire N, Kaur B, Naveed S, Sweep FC, Seckl MJ. Dutch risk classification and FIGO 2000 for gestational trophoblastic neoplasia compared. Poster presentation at the 19th International Meeting of the European Society of Gynaecological Oncology (ESGO), Nice, Frankrijk, October 25, 2016.

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Lieve tuinbewoners, bij jullie kon ik altijd aankloppen voor vragen, een luisterend oor of natuurlijk gewoon een break bij het koffie-apparaat. Maar nog veel belangrijker was natuurlijk alle gezelligheid en afleiding buiten de tuin. Van vrijmibo's (of ma-di-

wo-domibo's) tot waterskigekte. Van GPS tochten tot oma op het erf (Jawél het moét echt hier ergens zijn) tot een jengelend kolfapparaat in de skilift (wat rammelt er toch zo?) en natuurlijk Lissabon! 'Latin's from the North' die trip kostte me een jaar van mijn leven, maar het was het waard!

Beste gynaecologen, arts-assistenten, verloskundigen en verpleegkundigen uit het Jeroen Bosch Ziekenhuis. Hoewel ik ruim 3 jaar geen patiënt meer had gezien voelde ik me bij jullie vanaf dag 1 op mijn gemak. De sfeer bij jullie is echt goed, fijn dat ik daar even deel van mocht uitmaken!

Beste gynaecologen, arts-assistenten, verloskundigen en verpleegkundigen uit het Rijnstate Ziekenhuis. Dit is waar het allemaal begon! Wat heb ik een geweldig leuke tijd bij jullie gehad en ontzettend veel geleerd. Bovendien is door mijn laatste jaar 'chef Observatorium' geen consultendienst meer te gek! Ot, Wien klaar om weer eens onze toko te runnen? Naast veel mooie momenten, was er ook heftige casuïstiek, dank voor jullie vertrouwen en ik kijk er ontzettend naar uit om op 1 Januari 2018 weer bij jullie terug te mogen komen voor het begin van mijn opleiding tot gynaecoloog.

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Samsonmaatjes van het eerste uur, met natuurlijk als primair doel Ruud en Foen's jachtavontuur.

Als je niet zou weten dat onze menu's meestal uit blonde biertjes of trappist zouden bestaan, zou je haast denken dat we culinair aangelegd waren. Al is er aan happen ook nooit een tekort (Kaasplank, nacho's met dubbele saus, worstjes, bitterballen en had ik al kaasplank gezegd?). Foen, Rudie, Mona, Maarten, Lex, Loekie en natuurlijk ook mijn 'Broers' en alle anderen… dank voor jullie gezelligheid, luisterend oor en al die (soms ook doordeweekse) katers!

En dan alle reismaatjes die ik over de jaren heb verzameld! Niks werkt zo goed als een 'dit-moet-af-want-ik-ga-bijna-op-reis-deadline'. En die deadlines heb ik veel gehad de laatste jaren! Michelle, van Australië tot Afrika, met jou heb ik bijna alle 'hoeken' van de wereld gezien. Een kapotte auto duwen in het midden van een wildpark, maar ook

verplicht water drinken per order dokter Michelle als de Dengue heeft toegeslagen. Lieve Michelle, zullen we over een paar jaar maar eens voor de reüniereis met koters gaan? Lieve Mieke, want ben je toch een heerlijk nuchtere chick. Maar ondertussen schuiven de bedjes netjes tegen elkaar als er kleine beestjes op de loer kunnen liggen.. 'gezellig hè, ik heb jouw klamboe even over ons bed gehangen'. OpieJopie, jouw Afrika-liefde was allang op me overgeslagen. Maar bij jullie kunnen we ook altijd terecht voor een biertje (al moest ik de 0% zelf meenemen), of een knuffel voor Thomas. Betere buurtjes kunnen we ons denk ik niet wensen! Lieve loekie, wie is er nou net zo gek als ik om tijdens een reis te gaan sporten in de woestijn, op het strand of rond te springen door de hotelkamer. Stuiterbal, wanneer gaan we weer?

Lieve Iris, Anne en Josanne, lieve geneeskundecrew van het eerste uur, al zien we elkaar soms maanden of zelfs een jaar niet, we pakken gewoon de draad weer op. Dat zit wel goed! Lieve schone familie, Tiny en Hennie onze kleine kletskous voelt zich altijd enorm op zijn gemak bij jullie (dat geklets heeft hij natuurlijk niet van mij hoor). Dank, voor al jullie goede zorgen en liefde voor ons en ook de kleine man. De deur staat altijd open voor een bordje eten, een babyfoon of gewoon een goed glas wijn. Robin en Rikkie die Thailand-date halen we nog in!

En dan natuurlijk mijn lieve paranimfen, hoe de organisatie van een assistentenweekend zo uit de hand heeft kunnen lopen! Lieve Thijsie, wat moet ik altijd lachen om jouw directheid. Al heb ik nog wat extra respect voor Karin als je na wat biertjes je grenzen altijd nét weer even weet te verleggen. Lieve Lien, Pien, Mien (hoeveel bijnamen heb je eigenlijk?), al bijna 6 jaar collega's, van Rijnstate tot onderzoekstuin en terug! Ik waardeer jouw nuchterheid, je droge humor en natuurlijk je goede smaak voor bier! Lieve paranimfen, zelfs met een karmeliet in de ene hand konden jullie samen prima Thomas voeden met de andere, jullie zijn een fantastisch team!

En dan niet te vergeten natuurlijk ons fijne en inmiddels ook flink uitgebreide gezin! Bij ons in huis moet je toch echt wel je mannetje staan anders wordt er niet alleen niet naar je geluisterd, maar krijg je ook geen eten! (Jan en Mark jullie hebben daar inmiddels ook je draai wel in gevonden!). Lieve zusjes, Lieve Ragnhil en Steaffanne, we zijn heel verschillend, maar toch ook weer zo hetzelfde. Wat zijn jullie ontzettend gezellig, maar vooral ook lief! Thomas heeft het in zijn bedje soms nog over jullie! Lief

broertje van 1 meter 90, lieve Friedie, dank voor jullie hulp bij het zorgvuldig doorlezen van het hele manuscript. Wanneer gaan we weer eens bubbelen met cocktails?

Lieve mams, wat laat je jezelf en ons soms toch weer schrikken, maar sterk als je bent, klim je er altijd weer bovenop. Het voelde, maar was natuurlijk ook ver, heel ver weg toen ik in Australië de berichten van thuis ontving. Er zijn er maar weinig die zo vaak een ziekenhuisbed hebben aangetikt en toch zo vrolijk en positief in het leven staan. Ik ben blij dat je weer zo snel de oude bent, we kunnen je nog lang niet missen. Lieve paps, van grasmaaien naar schilderen, van koken naar beton storten en dat allemaal binnen een dag (of allemaal de helft). Wat heb jij nog een energie. Ik waardeer je interesse, je oog voor detail en ook de van jou geërfde liefde voor speciaalbier. Een oprecht trotse papa en opa, dank je wel! En tot slot natuurlijk ook Kiyon, Thyrza, Tayte en Stian, achtbanen, dierentuinen, trampolines, zwembaden niks is ons te gek. Jullie zijn stuk voor stuk toppers!

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Lieve Jeremy, al had je soms echt geen idee wat dat promoveren nou allemaal inhield, of wat die klinische verhalen nou allemaal betekende, je hoorde mijn verhalen en frustraties altijd aan en kwam met de mooiste no-nonsense oplossingen. We hebben een drukke tijd achter de rug met afronden promotie, start nieuwe baan en daarnaast een klein mannetje met sterke wil, maar papa regelt dat allemaal wel. Liefde is ook elkaar alles gunnen en dat doe jij als geen ander. Zonder jouw ongezouten mening is het leven ook maar saai!

CURRICULUM VITAE

Yalck Eysbouts werd ops 8 Augustus 1983 geboren in het 'Sint Radboud Ziekenhuis' in Nijmegen. In 2002 behaalde zij haar Gymnasium diploma aan het Stedelijk Gymnasium te Nijmegen. Na twee keer uitgeloot te zijn voor de opleiding geneeskunde lagen de papieren voor start van de studie in Antwerpen klaar. Drie maal was echter scheepsrecht en hoewel die Belgische biertjes lonkten startte zij in 2004 met de studie geneeskunde aan de Radboud Universiteit in Nijmegen. Omdat ook de studie psychologie, gestart na uitloting bleef interesseren, combineerde zij uiteindelijk haar opleiding met de studie psychologie, waarvoor zij in 2008 haar bachelor klinische psychologie behaalde. In 2007-2008 was zij tevens lid van het algemeen bestuur voor studenten klinische psychologie 'Mirus' waarbij de activiteiten varieerden van evaluatie en reorganisatie van onderwijs tot het organiseren van borrels, kroegentochten en een studiereis naar Boedapest. Eind 2008 startte zij haar wetenschappelijke stage in het Nepean Hospital in Sydney, Australië onder begeleiding van Dr. A. Poulton en Dr. J. Draaisma. Deze oorspronkelijke studiereis groeide al snel uit tot een wereldreis van 7 maanden. In 2011 volgde een co-schap gynaecologie in het Horacio Oduber Hospital op Aruba, waar ze (in de weekenden soms nog met bikini onder het werkpak) veel nieuwe eilandbewoners ter wereld bracht. December 2011 startte ze als arts-assistent niet in opleiding (ANIOS) op de afdeling gynaecologie en obstetrie in het Rijnstate in Arnhem. Na naast het klinische werk onder begeleiding van gynaecologe Annemiek Nap van het onderzoeksleven geproefd te hebben startte zij op 1 Maart 2014 als arts-onderzoeker op het gebied van trofoblastziekten voor de afdelingen gynaecologie en obstetrie, laboratoriumgeneeskunde en medische oncologie in het Radboudumc te Nijmegen (promotoren Prof. dr. LFAG Massuger, Prof dr. FCGJ Sweep en co-promotoren dr. PB Ottevanger en dr. CMG Thomas). In Januari 2018 zal zij in Arnhem starten aan haar opleiding tot gynaecoloog. Yalck is getrouwd met Jeremy Roos, samen hebben zij een zoon van 2, Thomas.